Available online at www.sciencedirect.com

SCIENCE DIRECT

Biochimica et Biophysica Acta 1658 (2004) 157–164

BIOCHIMICA ET BIOPHYSICA ACTA BBA

www.bba-direct.com

Review

Activation by retinoids of the uncoupling protein UCP1

Paula Tomás[a], Jesús Jiménez-Jiménez[a], Pilar Zaragoza[a], Vidyasagar Vuligonda[b], Roshantha A.S. Chandraratna[b], Eduardo Rial[a,*]

[a] *Centro de Investigaciones Biológicas, CSIC, Ramiro de Maeztu 9, E-28040 Madrid, Spain*
[b] *Division of Retinoid Research, Allergan Inc., 2525 Dupont Drive, Irvine, CA 92612, USA*

Received 8 April 2004; received in revised form 17 May 2004; accepted 17 May 2004
Available online 11 June 2004

Abstract

The uncoupling protein from brown adipose tissue (UCP1) is a transporter that catalyzes a regulated discharged of the mitochondrial proton gradient. The proton conductance in UCP1 is inhibited by nucleotides and activated by fatty acids. We have recently shown that all-*trans*-retinoic acid (ATRA) is a high-affinity activator of UCP1. In the present report, we have set to analyze the structural requirements for the ligands that activate UCP1 and particularly the specificity for different retinoids. For this purpose, we have developed a new protocol to determine the activity of UCP1 in respiring yeast mitochondria that can be adapted for high-throughput screenings. Our results evidence differences between the structural requirements for the activation by fatty acids and retinoids. Thus, although all active retinoids must possess a carboxylate, the introduction of additional polar groups renders them inactive. The linear and rigid structure of these molecules suggests the existence of a long hydrophobic binding pocket. We postulate that the access to the retinoid binding site must occur from the lipid bilayer and this could be at the interface between two transmembrane α-helices.

Keywords: Uncoupling protein; Retinoid; Screening; Respiration; Mitochondrion; Yeast

1. Introduction

The uncoupling protein from brown adipose tissue (UCP1) is a transporter of the mitochondrial inner membrane whose function is the dissipation of the proton gradient. The activity of UCP1 is tightly regulated. Purine nucleotides bind to UCP1 from the cytosolic side and inhibit the proton transport activity. Fatty acids can override the nucleotide inhibition and increase the proton conductance. The effects of these two regulatory ligands match with the known events that take place during the physiological activation of thermogenesis in the brown adipocyte. The hypothalamus signals the initiation of thermogenesis through the sympathetic nervous system. Noradrenaline binds to β-receptors and initiates a cAMP-dependent lipolytic cascade. The fatty acids liberated serve two functions: they are the substrate that mitochondria will oxidize but also the activators of UCP1. When the hormonal stimulation ceases, lipolysis stops and once fatty acids are oxidized, UCP1 will return to the nucleotide-inhibited state (reviewed in Ref. [1]). We have recently described that all-*trans*-retinoic acid (ATRA) is a high-affinity activator of UCP1 [2]. Interestingly, ATRA is also a powerful transcriptional activator of the *ucp1* gene and the concentration required to increase the protein's activity is within the range that promotes gene expression [3]. Therefore, this regulatory pathway could also be of physiological relevance.

Retinoids are generally defined as natural or synthetic derivatives of vitamin A. The natural occurring retinoids play important roles in a variety of biological processes such as cell growth and differentiation, immune response, coagulation, etc. Retinoids exert their action by binding to nuclear receptors that act as ligand-activated transcription factors (reviewed in Ref. [4]). Additionally, certain retinoids have been shown to induce apoptosis through mechanisms that seem independent of the nuclear receptors (reviewed in Ref. [5]). Numerous synthetic retinoids have been developed with therapeutic aims. Thus, for example, retinoid-based therapies are being used for the treatment of certain

Abbreviations: ATRA, all-*trans*-retinoic acid; UCP, uncoupling protein

* Corresponding author. Tel.: +34-91-837-3112x4236; fax: +34-91-536-04-32.
E-mail address: rial@cib.csic.es (E. Rial).

0005-2728/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2004.05.010

types of cancers or skin disorders (reviewed in Ref. [6]). The description of the activation of UCP1 and UCP2 by ATRA [2] has opened the door to the possible use of retinoids to modulate the energetic efficiency at the mitochondrial level.

The yeast *Saccharomyces cerevisiae* has been used over the last decade to investigate the transport properties of recombinantly expressed UCP1 [7–9]. Since the discovery of the new UCPs, *S. cerevisiae* has again been chosen by several groups to investigate their bioenergetic properties [10–12]. However, caution should be exerted when interpreting results obtained with the new UCPs in recombinant systems [13]. In the present report, we present a fluorescent method to determine the rate of respiration in isolated yeast mitochondria that is applied to search for regulators of the UCP1. The screening with a number of organic acids and retinoids has provided valuable information on the transport mechanism, the requirements for compounds to be active and the nature of their binding site.

2. Materials and methods

The procedures for recombinant expression of the rat uncoupling protein UCP1 in the *S. cerevisiae* strain W303 have been described previously [7]. The control strain contained the same vector but with the UCP1 cDNA in the inverse orientation. Yeast growth and mitochondrial isolation protocols have been described previously [7]. Protein was determined by the Lowry method using albumin as standard.

The rate of mitochondrial respiration has been measured using two techniques. The classic procedure uses a Hansatech oxygen electrode (Hansatech Instruments, King's Lynn, England). Mitochondria (0.15 mg protein/ml) were incubated at 20 °C in medium containing 0.65 M mannitol, 0.5 mM EGTA, 10 mM phosphate, 2 mM $MgCl_2$, 1 mg/ml essentially fatty-acid-free albumin and 10 mM Tris/maleate pH 6.8. Respiration was initiated by the addition of 1 mM NADH. The second procedure is based on the monitorship of the fluorescence decrease during the mitochondrial oxidation of NADH. Experiments were performed on a standard uncoated 96-well microtiter plate (Nunc, Denmark). Conditions were the same as for the oxygen electrode experiments except that the concentration of NADH was 0.6 mM and the protein concentration 0.1 mg/ml. Regulators (GDP, palmitate, retinoids, etc.) were added to the wells before the NADH addition. Plates were read on a POLARstar Galaxy microplate reader (BMG Labtechnologies, Offenburg, Germany). Excitation was set at 340 nm and emission at 470 nm. To ensure homogeneity of the mitochondrial suspensions, plates were shaken for 10 s at the end of each reading cycle.

The retinoids 4-[(*E*)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphtalenyl)-1-propenyl] benzoic acid (TTNPB) and 4-[(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphtalenyl)carboxamido] benzoic acid (AM580) were from Tocris (Langford, UK). All other retinoids were synthesized at Allergan and their structures shown in Fig. 2. Other organic compounds tested were from Aldrich (Gillingham-Dorset, UK) or Lancaster Synthesis (Morecambe, UK). All other reagents were from Sigma (St. Louis, USA).

3. Results and discussion

3.1. Screening protocol

Recombinant expression of the UCPs in yeasts and the subsequent isolation of mitochondria has been widely used to study their bioenergetic properties. An alternative procedure is the use of proteins reconstituted into liposomes. The main advantage of the first system lies in the possibility of performing studies in the presence of a high and stable membrane potential that resembles closer the in vivo conditions.

Plant and yeast mitochondria present a distinct feature from mammalian mitochondria, namely their ability to oxidize cytosolic NAD(P)H. NAD(P)H presents an absorption band at 340 nm that disappears when the coenzymes are oxidized. Additionally, the light absorbed at 340 nm is emitted as fluorescent light with a maximum at 460 nm. Therefore, changes in absorption or fluorescence can be used to follow variations in the redox state of these coenzymes. We have used this approach to measure the rate of respiration of *S. cerevisiae* mitochondria and determine the activity of UCP1. Thus, Fig. 1 shows the effects of nucleotides and fatty acids on UCP1 mitochondria (A and C) and are compared with those on control mitochondria (B and D). The fluorescence data (A and B) can be converted to NADH concentration values (C and D) after signal calibration. The slope of the regression lines are the rates of NADH consumption and these values are summarized in Table 1.

This methodology confirms the known bioenergetic properties of UCP1. Thus, basal respiration is higher for mitochondria from UCP1 expressing yeasts than for control (Table 1) and this difference reflects the basal proton transport activity of UCP1. This UCP1-dependent proton flux can be inhibited with GDP and then the respiratory rate becomes indistinguishable from that of control yeasts. As expected, the rate of fluorescence decrease is faster in the presence of palmitate and can be inhibited with GDP. Table 1 also shows that there is a perfect agreement between of the data generated with the microplate reader and those obtained with the oxygen electrode.

3.2. Activation of UCP1 by organic acids

Fatty acids activate the uncoupling protein UCP1 but the molecular mechanism has remained controversial for a long time [14]. Two models have been put forward to explain the activity of UCP1 where fatty acids operate as either

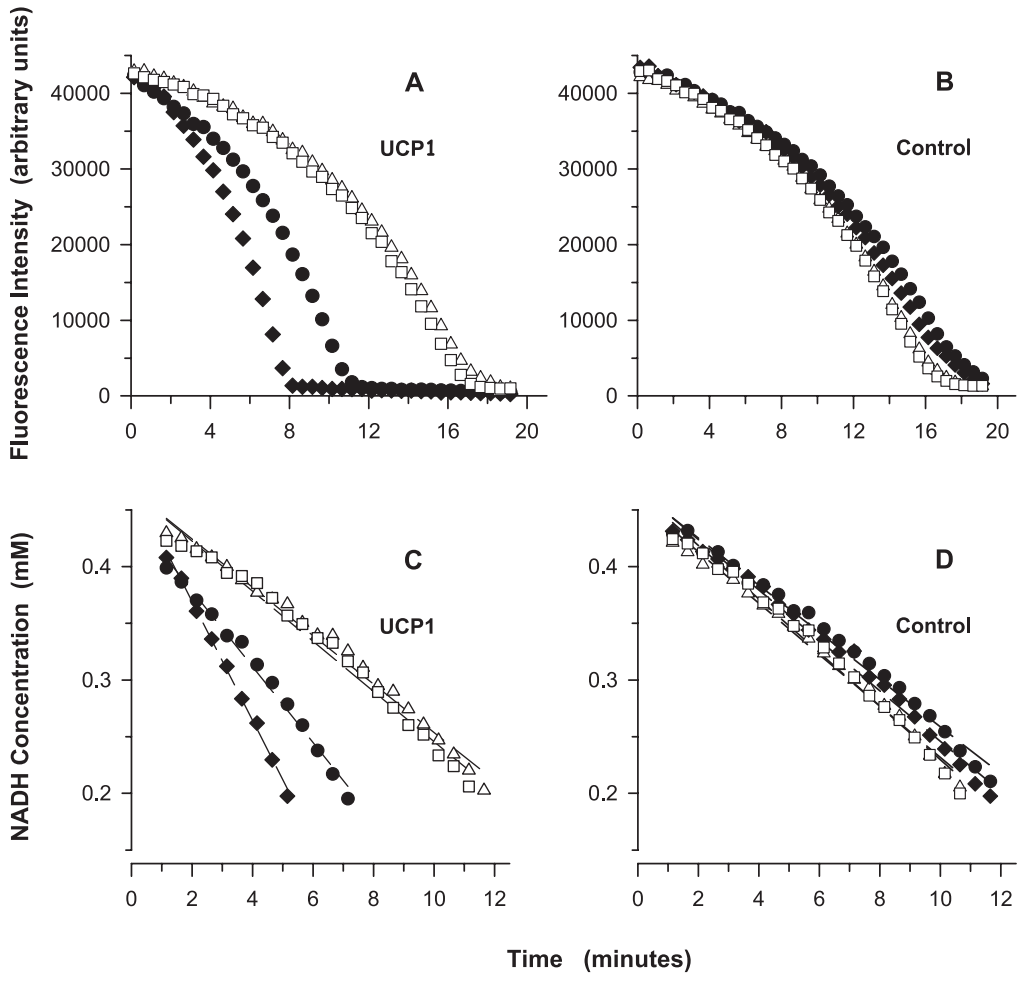

Fig. 1. Analysis with a microplate reader of the regulation of the uncoupling protein UCP1. (A and B) Fluorescence decrease; (C and D) NADH consumption. Panels A and C show the analysis on mitochondria from UCP1 expressing yeasts while panels B and D correspond to control yeasts. (●) Basal respiration; (♦) 48 μM palmitate present; (□) 1 mM GDP present; (△) in the presence of 48 μM palmitate and 1 mM GDP. Dashed lines in panels C and D represent the linear regression and the slopes are summarized in Table 1. Data are the average of the readings of three plate wells.

substrate (“fatty acid cycling hypothesis”) or prosthetic group (“proton buffering model”). The first hypothesis proposes the existence of a cycle where the protonated fatty acid would flip-flop in the membrane to deliver a proton to the matrix side and UCP1 would catalyze the return of the fatty acid anion to the cytosolic side of the membrane. In the alternative mechanism, fatty acids act as a prosthetic group in UCP1: the carboxylate would bind protons and deliver them to a site from which they are translocated to the other side of the membrane. We have recently demonstrated that undecanosulfonate can activate UCP1 [14] and since liposome studies have shown that alkylsulfonates cannot

Table 1
Comparison of the rates of respiration of yeast mitochondria measured with an oxygen electrode or a fluorescence microplate reader

	UCP1 Mitochondria		Control Mitochondria	
	Oxygen consumption[a]	NADH consumption[b]	Oxygen consumption[a]	NADH Consumption[b]
Basal respiration	218	223	144	141
Uncoupled respiration	773	675	763	691
GDP 3 mM	149	142	155	151
Palmitate 48 μM	337	349	157	148
Palmitate 48 μM + GDP 3 mM	140	147	157	157

Determinations were performed with the same mitochondrial preparations. Oxygen consumption measurements were performed in duplicate while NADH oxidation rates are the average of values obtained with three plate wells. Uncoupled respiration was determined in the presence of 10 μM FCCP.

[a] Values in nanomoles of ‘O’ consumed per minute and per milligram of protein. The oxygen content at 20 °C is considered to be 284 nmol/ml.

[b] Values in nanomoles of NADH consumed per minute and per milligram of protein.

flip-flop [15], it implies that the fatty acid cycling model cannot explain the transport mechanism.

The investigation of the specificity of the UCP1 activators could also help to unravel the mechanism of activation. The structural requirements for the activators do not appear to be very stringent; thus, compounds with a free carboxylate group and with sufficient lipid solubility will increase its proton conductance [16–19]. There are, however, discrepancies in the data available. A second carboxylate in the ω position seems tolerated in the hands of some authors [19] but not others [18]. A similar situation is found with the presence of aromatic rings in the molecule. There is a consensus on the acceptance of bulky groups along the aliphatic chain but there is disagreement if they are present at the end of the tail. Thus, Jezek et al. [18] have shown that 4-heptyl-benzoic increases the UCP1-mediated proton transport in liposomes while 6-phenyl-hexanoic did not. However, Klingenberg and Huang [19], also working with proteoliposomes, see a moderate proton transport with 6-phenyl-hexanoic but it is markedly increased when 8-phenyl-octanoic is used. We have also reported that ATRA is a strong activator despite the presence of a planar-bulky structure far from the carboxylate [2].

To investigate the structural requirements of aromatic compounds to activate UCP1 further, we have used the new screening protocol that relies on measurements under respiring conditions. Fig. 2 presents the structural formula of the compounds tested and Fig. 3 the results of the screening. As we have previously reported [2] ATRA presents a high affinity although the kinetics shows saturation with a maximum respiratory activity lower than that induced by palmitate. Compounds that elicit a respiration increase lower

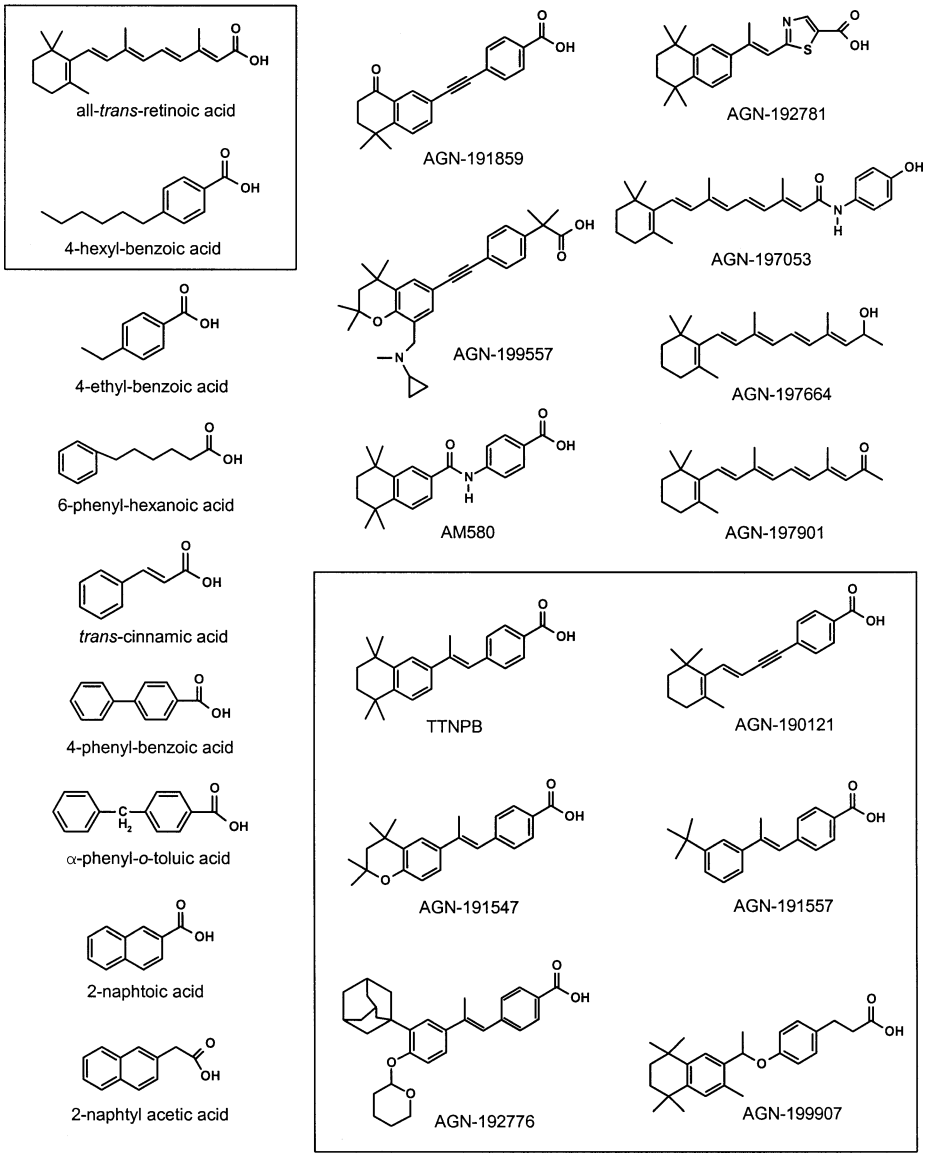

Fig. 2. Structure of the compounds tested. Compounds that activate UCP1 are included in the boxes.

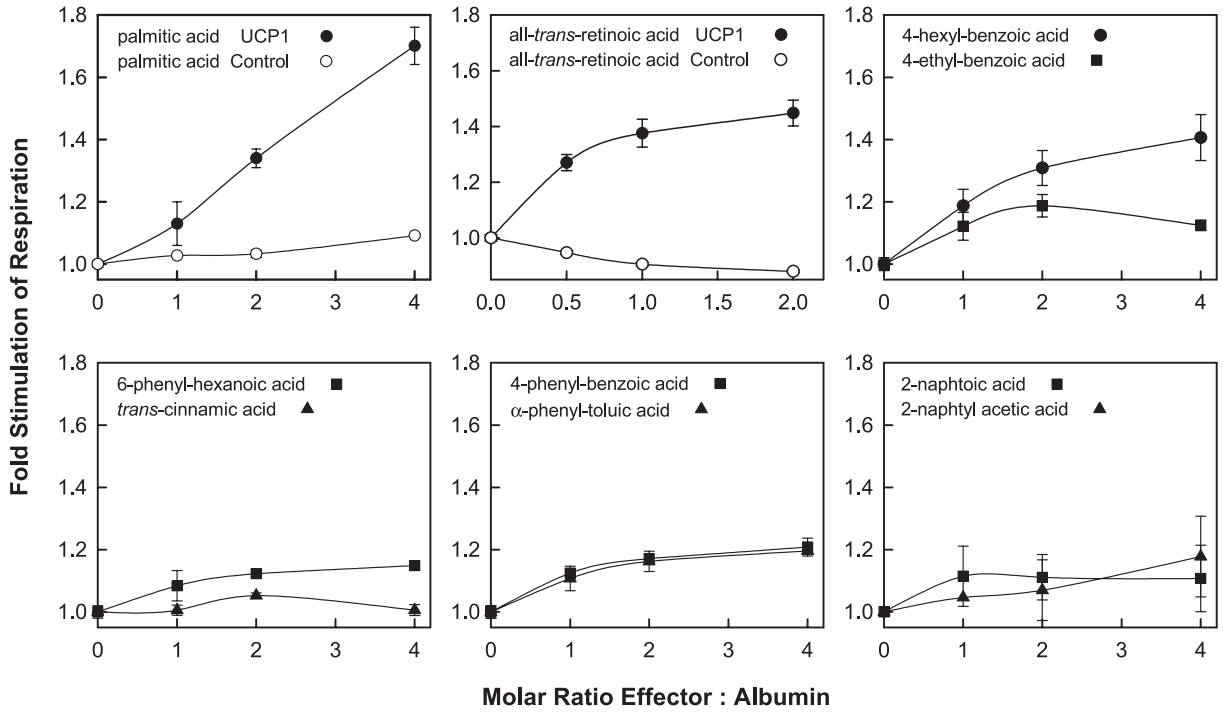

Fig. 3. Activation of UCP1 by organic acids. The two top panels present the effect of palmitate (left) and ATRA (right) on control mitochondria (○) and mitochondria expressing UCP1 (●). In the rest of the panels, only the effects on UCP1 mitochondria are shown. Albumin concentration was 1.6 μM. Data points represent the mean ± S.E. of two to three independent experiments performed at least in duplicate.

than 20% are considered inactive since this type of compounds generally causes a 10–20% increase in respiration in control mitochondria (data not shown). The results obtained with these organic compounds are in good agreement with those published by Jezek et al. [18]. Thus, for example in our system 4-hexyl-benzoic is active while 6-phenyl-hexanoic is not. Interestingly, the kinetics of hexyl-benzoic resembles that of retinoic acid. A number of other compounds with benzene rings distant from the carboxylate were also inactive. Our screening protocol also portrays *trans*-cinnamic acid as an inactive molecule and therefore contradicts the observations reported by Echtay et al. [20].

3.3. Activation of UCP1 by retinoids

The activation of UCP1 by ATRA would seem puzzling in view of the structure of the activators shown in Fig. 3 and in Ref. [18]. Since a large collection of compounds structurally related to retinoic acid have been synthesized as specific ligands for retinoid receptors, we decided to screen a set of these compounds to get an insight into the structural requirements of the activators of the UCP1 and their binding site. A first selection of compounds had to be made by analyzing their effects on control yeast mitochondria. Our screening protocol was particularly useful for this task. A significant proportion of the retinoids could not be used to investigate their activity as regulators of UCP1 because of their unspecific effects on control mitochondria. Thus, some compounds showed uncoupling activity, others were inhibitors of respiration and some retinoids showed these two effects depending on their concentration (data not shown). Data collected in Fig. 4 are for those compounds that gave unequivocal effects on yeast mitochondria expressing UCP1. Thus, for example, ATRA (Fig. 3) or AGN 191547 (Fig. 4) causes a mild inhibition of respiration in control yeasts but activation of UCP1 can be clearly established. Similarly, the uncoupling activity of AGN 193776 or AGN 199907 could be easily differentiated from the effects on UCP1. The rate of respiration in the presence of GDP (Fig. 5) also reflects the UCP-independent activity; hence, the smaller bars in ATRA, TTNPB or AGN 181547 confirm the inhibition of respiration seen in control mitochondria. In fact, the GDP-sensitive component (difference between the two bars) is a more realistic measure of the relative activity of the retinoids. Nevertheless, the distinction between active and inactive retinoids for this set of compounds is evident.

The first observation that should be pointed out is that when the carboxylate group of the retinoid was replaced by a hydroxyl or carbonyl group (AGN 197053, AGN 197664 or AGN 197901), the compounds are inactive. The need for a free carboxylate was pointed out 20 years ago after the analysis with fatty acid derivatives [16]. This requirement was confirmed by other authors [17,18] and has been interpreted as an indication of the involvement of the carboxylate in proton translocation, no matter the regulatory model invoked. This notion has recently been challenged by Echtay et al. [20] who reported that retinal or 4-hydroxy-nonenal

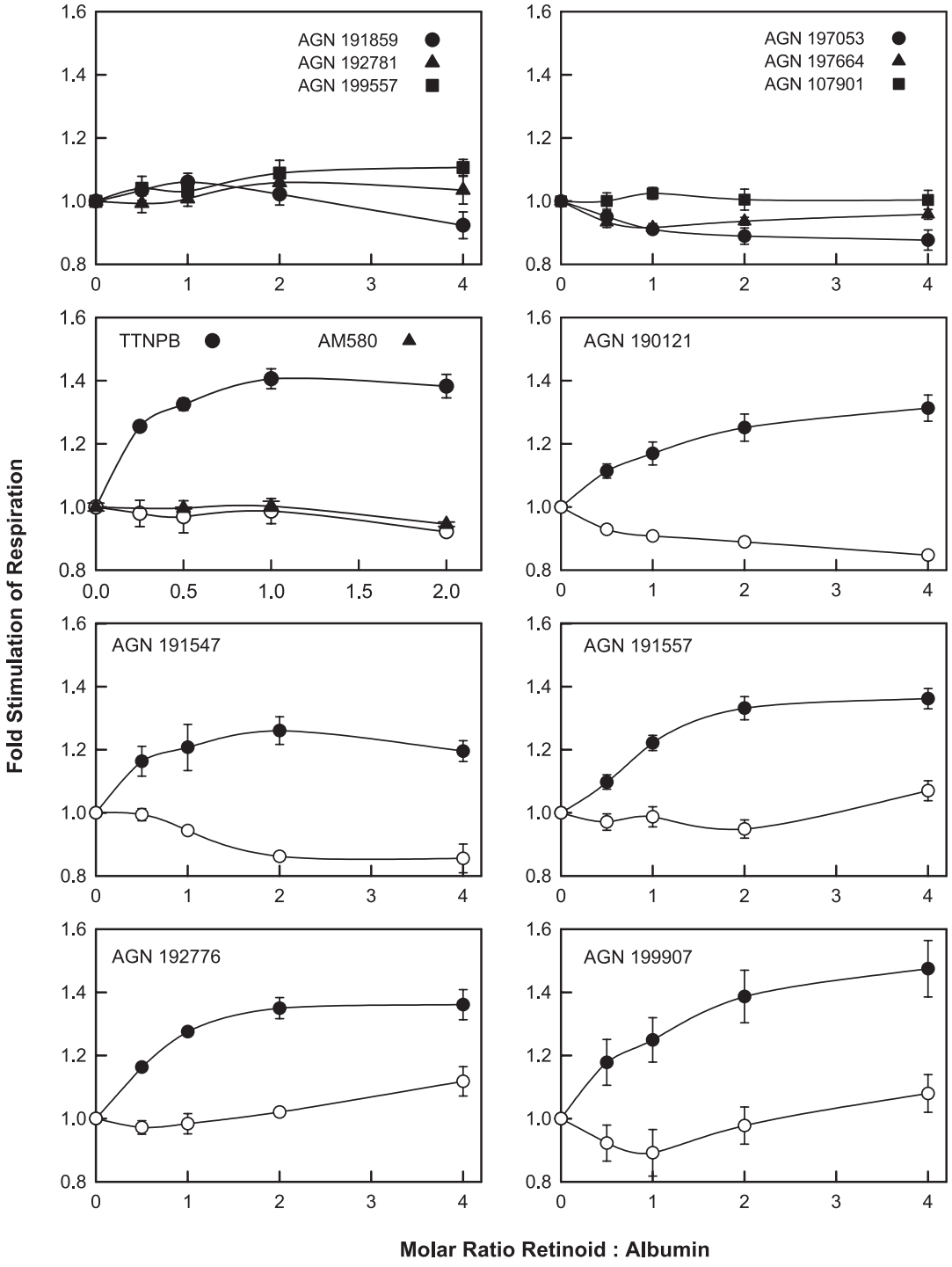

Fig. 4. Activation of UCP1 by retinoids. In all cases, filled symbols (●, ■, ▲) correspond to the data obtained with mitochondria from UCP1 expressing yeasts and empty circles with control yeast mitochondria. Albumin concentration was 1.6 μM. Data points represent the mean ± S.E. of two to three independent experiments performed at least in duplicate.

activates uncoupling mediated not only by UCP1 but also by UCP2, UCP3 and the ADP/ATP carrier. These authors suggest that the activation by these compounds is due to the reaction of the functional group 2-alkenal with specific residues in the UCPs or the ADP/ATP carrier. This mechanism cannot be invoked for the activation of UCP1 in brown fat where fatty acids are the physiological regulators and this functional group is not present in them.

The second feature that should be highlighted concerns the effect of polar groups in the retinoid molecule. The analysis of the fatty acid specificity revealed a remarkable tolerance to the presence of hydrophilic substituents if a

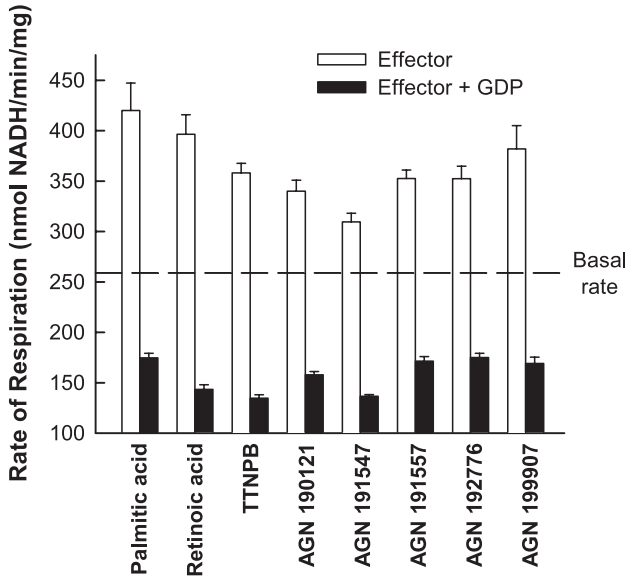

Fig. 5. Nucleotide sensitivity of the activation of UCP1 by retinoids. Bars represent the mean ± S.E. of two to three independent experiments performed at least in duplicate. The dashed horizontal line represents the basal rate of respiration and it represents the mean value of the respiratory rates in the absence of the effectors. The concentration of the different retinoids was the highest presented in Fig. 4. Empty bars represent the rates of respiration in the presence of retinoid while filled bars are the rates obtained at the same retinoid concentration but in the presence of 3 mM GDP.

sufficient lipid solubility was preserved [17,18]. The data provided by the screening with the retinoids points to a different situation since the presence of polar groups is not tolerated. One good example is the comparison between TTNPB and AM580 (see structures in Fig. 2). TTNPB is the retinoid that shows the highest affinity and the substitution of the central propenyl by an amide (AM580) leads to the complete loss of activity. A similar conclusion can be drawn after the comparison of AGN 190121 and AGN 191859 where the substitution of two methyl groups by a carbonyl also leads to an inactive retinoid.

The activation of UCP1 by retinoids starts to provide information on their binding site. Most of the active retinoids presented in Fig. 2 are extended molecules with little flexibility that would require the presence of a binding pocket with a length of at least 15 Å. The clear hydrophobic profile of these compounds would suggest the access to the binding site from the lipid bilayer. We have previously reported that photoaffinity labelling of UCP1 with ATRA is increased in the presence of ubiquinone [21]. Ubiquinone has been postulated as an essential cofactor for the activity of UCP1 [22] although this view has been challenged [23,24]. We can envisage that ubiquinone facilitates or participates in binding of the retinoids to UCP1 and that this binding occurs by lateral diffusion from the lipid phase of the membrane.

The 3D structure of the UCP1 is not known. We have presented a model for the transmembrane arrangement of the UCP1 that proposes the existence of an α-helix bundle formed by the six transmembrane domains that spans the hydrophobic core of the membrane to form the main translocation pathway [25]. This arrangement has recently been confirmed with the publication of the 3D structure of the ADP/ATP carrier [26]. We now postulate that this retinoid binding pocket could be located at the interface between two transmembrane α-helices. The establishment of the tight binding of the retinoids, deduced from their high affinity, would suggest a predominance of strong hydrophobic interactions and therefore an exclusion of compounds that present marked polar residues in their structure.

The final question concerns the relation between the retinoid binding site and the fatty acid binding site. We have previously reported that the activation effects of ATRA and palmitate are neither synergistic nor additive and that, therefore, they must be acting on the same site [2]. These new data suggest that the two types of ligands must differ in their mechanism of binding although the functional carboxylate group may end up in the same site to fulfill its regulatory function. Indeed, it is striking, first, the difference in the activation kinetics that for all retinoids saturating with a V_{max} markedly lower than that of fatty acids and, secondly, the difference in the tolerance for hydrophilic substituents.

4. Concluding remarks

We have demonstrated that the use of a microplate reader is well suited to follow the respiration of yeast mitochondria. We have applied this methodology to screen for compounds that activate UCP1. For the present assays, a 96-well microtiter plate has been used with 120 μl per well. Since the molar extinction coefficient and the quantum yield of the NADH fluorescence are very high, assay volumes can be decreased markedly without compromising the quality of the signal. Similarly, the protein concentration can also be decreased significantly if the reading time is increased accordingly. These two features are an indication that the procedure can be easily adapted to the requirements of a high throughput screening protocol.

We have reported that undecanosulfonate activates proton transport in UCP1 and since this compound cannot flip-flop, the fatty acid cycling hypothesis cannot be the mechanism that explains the increased membrane proton conductance [16]. The flip-flop capacity of different organic acids has been investigated in liposomes [27]. These studies revealed that compounds with aromatic rings, such as 6-phenyl-hexanoic, biphenyl-2-carboxylic, 2-naphtoic or 3,3-diphenyl-propionic, were unable to flip-flop. It has been argued that this could be due to the bulkiness and planar structure of the benzene ring [27]. Some active retinoids used in our study present structural features that would make unlikely their flip-flop in the membrane and, therefore, it is an additional argument against the fatty acid cycling hypothesis.

Acknowledgements

The authors wish to thank Dr. Javier Cañada for his advice and discussions. This work is supported by Allergan Sales Inc. and a project grant from the Spanish Ministry of Science and Technology (BIO2002-00142) to E.R. P.T. was supported by Allergan.

References

[1] D.G. Nicholls, R.M. Locke, Thermogenic mechanisms in brown fat, Physiol. Rev. 64 (1984) 1–64.
[2] E. Rial, M.M. González-Barroso, C. Fleury, S. Iturrizaga, D. Sanchis, J. Jiménez-Jiménez, D. Ricquier, M. Goubern, F. Bouillaud, Retinoids activate proton transport by the uncoupling proteins UCP1 and UCP2, EMBO J. 18 (1999) 5827–5833.
[3] R. Alvarez, J. De Andrés, P. Yubero, O. Viñas, T. Mampel, R. Iglesias, M. Giralt, F. Villarroya, A novel regulatory pathway of brown fat thermogenesis. Retinoic acid is a transcriptional activator of the mitochondrial uncoupling protein gene, J. Biol. Chem. 270 (1995) 5666–5673.
[4] P. Chambon, A decade of molecular biology of retinoic acid receptors, FASEB J. 10 (1996) 940–954.
[5] M. Pfahl, F.J. Piedrafita, Retinoid targets for apoptosis induction, Oncogene 22 (2003) 9058–9062.
[6] S.M. Thacher, J. Vasudevan, R.A.S. Chandraratna, Therapeutic applications for ligands of retinoid receptors, Curr. Pharm. Des. 6 (2000) 25–58.
[7] I. Arechaga, S. Raimbault, S. Prieto, C. Levi-Meyrueis, P. Zaragoza, B. Miroux, D. Ricquier, F. Bouillaud, E. Rial, Cysteine residues are not essential for uncoupling protein function, Biochem. J. 296 (1993) 693–700.
[8] K.S. Echtay, M. Bienengraeber, M. Klingenberg, Mutagenesis of the uncoupling protein of brown adipose tissue. Neutralization of E190 largely abolishes pH control of nucleotide binding, Biochemistry 36 (1997) 8253–8260.
[9] M. Modriansky, D.L. Murdza-Inglis, H.V. Patel, K.B. Freeman, K.D. Garlid, Identification by site-directed mutagenesis of three arginines in uncoupling protein that essential for nucleotide binding and inhibition, J. Biol. Chem. 272 (1997) 24759–24762.
[10] C. Fleury, M. Neverova, S. Collins, S. Raimbault, O. Champigny, C. Levi-Meyrueis, F. Bouillaud, M.F. Seldin, R.S. Surwit, D. Ricquier, C.H. Warden, Uncoupling protein-2: a novel gene linked to obesity and hyperinsulineamia, Nat. Genet. 15 (1997) 269–272.
[11] D. Sanchis, C. Fleury, N. Chomiki, M. Goubern, Q. Huang, M. Neverova, F. Grégorie, J. Eastlick, S. Raimbault, C. Lévi-Meyrueis, B. Miroux, S. Collins, M. Seldin, D. Richard, C. Warden, F. Bouillaud, D. Ricquier, BMCP1, a novel mitochondrial carrier with high expression in the central nervous system of humans and rodents, and respiration uncoupling activity in recombinant yeast, J. Biol. Chem. 273 (1998) 34611–34615.
[12] T. Hagen, C.-Y. Zhang, L.J. Slieker, W.K. Chung, R.L. Leibel, B.B. Lowell, Assessment of uncoupling activity of the human uncoupling protein 3 short form and three mutants of uncoupling protein gene using a yeast heterologous expression system, FEBS Lett. 454 (1999) 201–206.
[13] D. Heidkaemper, E. Winkler, V. Müller, K. Frischmuth, Q. Liu, T. Caskey, M. Klingenberg, The bulk of UCP3 expressed in yeast cells is incompetent for a nucleotide regulated H^+ transport, FEBS Lett. 480 (2000) 265–270.
[14] E. Rial, E. Aguirregoitia, J. Jiménez-Jiménez, A. Ledesma, Alkylsulfonates activate the uncoupling protein UCP1: implications for the transport mechanism, Biochim. Biophys. Acta 1608 (2004) 122–130.
[15] K.D. Garlid, D.E. Orosz, M. Modriansky, S. Vassanelli, P. Jezek, On the mechanism of fatty acid-induced proton transport by mitochondrial uncoupling protein, J. Biol. Chem. 271 (1996) 2615–2620.
[16] E. Rial, A. Poustie, D.G. Nicholls, Brown adipose tissue mitochondria: the regulation of the 32000 Mr uncoupling protein by fatty acids and purine nucleotides, Eur. J. Biochem. 137 (1983) 197–203.
[17] E. Winkler, M. Klingenberg, Effect of fatty acids on H^+ transport activity of the reconstituted uncoupling protein, J. Biol. Chem. 269 (1994) 2508–2515.
[18] P. Jezek, M. Modriansky, K.D. Garlid, A structure-activity study of fatty acid interaction with mitochondrial uncoupling protein, FEBS Lett. 408 (1997) 166–170.
[19] M. Klingenberg, S.G. Huang, Structure and function of the uncoupling protein from brown adipose tissue, Biochim. Biophys. Acta 1415 (1999) 271–296.
[20] K.S. Echtay, T.C. Esteves, J.L. Pakay, M.B. Jekabsons, A.L. Lambert, tero-Otin, M. Portero-Otin, R. Pamplona, A.J. Vidal-Puig, S. Wang, S.J. Roebuck, M.D. Brand, A signalling role for 4-hydroxy-2-nonenal in regulation of mitochondrial uncoupling, EMBO J. 22 (2003) 4103–4110.
[21] P. Tomás, A. Ledesma, E. Rial, Photoaffinity labeling of the uncoupling protein UCP1 with retinoic acid: ubiquinone favors binding, FEBS Lett. 526 (2002) 63–65.
[22] K.S. Echtay, E. Winkler, M. Klingenberg, Coenzyme Q is an obligatory cofactor for uncoupling protein function, Nature 408 (2000) 609–613.
[23] M. Jaburek, K.D. Garlid, Reconstitution of recombinant uncoupling proteins: UCP1, -2, and -3 have similar affinities for ATP and are unaffected by coenzyme Q10, J. Biol. Chem. 278 (2003) 25825–25831.
[24] T.C. Esteves, K.S. Echtay, T. Jonassen, C.F. Clarke, M.D. Brand, Ubiquinone is not required for proton conductance by uncoupling protein 1 in yeast mitochondria, Biochem. J. 379 (2004) 309–315.
[25] A. Ledesma, M. García de Lacoba, I. Arechaga, E. Rial, Modelling the transmembrane arrangement of the uncoupling protein UCP1 and topological considerations of the nucleotide-binding-site, J. Bioenerg. Biomembranes 34 (2002) 473–486.
[26] E. Pebay-Peyroula, C. Dahout-Gonzalez, R. Kahn, V. Trézéguet, G.J.M. Lauquin, G. Brandolin, Structure of mitochondrial ADP/ATP carrier in complex with carboxyatractyloside, Nature 426 (2003) 39–44.
[27] P. Jezek, M. Modriansky, K.D. Garlid, Inactive fatty acids are unable to flip-flop across the lipid bilayer, FEBS Lett. 408 (1997) 161–165.