New molecular aspects of regulation of mitochondrial activity by fenofibrate and fasting

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Abstract Fenofibrate and fasting are known to regulate several genes involved in lipid metabolism in a similar way. In this study measuring several mitochondrial enzyme activities, we demonstrate that, in contrast to citrate synthase and complex II, cytochrome *c* oxidase (COX) is a specific target of these two treatments. In mouse liver organelles, Western blot experiments indicated that mitochondrial levels of p43, a mitochondrial T3 receptor, and mitochondrial peroxisome proliferator activated receptor (mt-PPAR), previously described as a dimeric partner of p43 in the organelle, are increased by both fenofibrate and fasting. In addition, in PPARα-deficient mice, this influence was abolished for mt-PPAR but not for p43, whereas the increase in COX activity was not altered. These data indicate that: (1) PPARα is involved in specific regulation of mt-PPAR expression by both treatments; (2) fenofibrate and fasting regulate the mitochondrial levels of p43 and thus affect the efficiency of the direct T3 mitochondrial pathway.

Key words: Mitochondrion; Fibrate; Fasting; Mitochondrial T3 receptor; Peroxisome proliferator activated receptor

1. Introduction

β-Oxidation is a major process by which fatty acids are oxidized. From this reaction, mitochondria produce most of the energy in animal cells through the oxidative phosphorylation chain containing enzymatic subunits encoded by both nuclear and mitochondrial genes.

Several peroxisome proliferators (PPs) such as fibrate [1] have been shown to activate peroxisome proliferator activated receptors (PPARs) which are members of the steroid nuclear receptor superfamily [1,2]. PPs are known to activate enzymes involved in the regulation of lipid metabolism (peroxisomal and mitochondrial β-oxidation, microsomal ω-hydroxylation and ketone body synthesis) [2–7]. Several studies have also underlined that fasting, like PP exposure, induces β-oxidation gene expression, probably by increasing fatty acid levels [8,9]. Moreover, experiments performed in PPARα-deficient mice (PPARα−/−) established that this nuclear receptor is needed for the induction by fibrate or fasting of several genes involved in lipid oxidation such as acyl-CoA oxidase, enoyl-CoA hydratase and 3-ketoacyl-CoA thiolase [8–11]. These observations point to a key role for PPARα in lipid homeostasis.

Like thyroid hormone [12–17], PPs regulate mitochondrial activity [18–20]. They alter mitochondrial morphology and enzyme composition [21–23]. In addition, they also increase mitochondrial mRNA and rRNA levels [18]. Today, it has been proposed that PPs, such as fenofibrate, indirectly act at organelle level through the PPAR nuclear pathway.

In addition to the well-known mitochondrial transcription factor mt-TFA [24], we have previously demonstrated that a triiodothyronine receptor (p43) located in the mitochondrial matrix is a potent mitochondrial transcription factor [16,17]. Moreover, we have characterized a 45 kDa protein immunologically related to PPARγ2 (naming it mt-PPAR), occurring in the mitochondrial matrix [25]. Interestingly, mt-PPAR and p43 have been detected in a common complex which binds to a DR2 sequence of the mitochondrial D-loop [25].

In the present work, we have studied the influence of fenofibrate treatment or fasting on p43 and mt-PPAR mitochondrial levels in relation to mitochondrial activity. We also tested the possible involvement of the nuclear receptor PPARα in the regulation of these factors by using liver mitochondria extracted from control or PPARα-deficient mice. We report here that fenofibrate or fasting do not influence mt-TFA amounts but increase mitochondrial levels of p43 and mt-PPAR in a similar way. In addition, we found that PPARα is involved in the regulation of mt-PPAR expression.

2. Materials and methods

2.1. Animals

Fenofibrate treatment or fasting were carried out in control (C57BL/6) or PPARα-deficient mice on a C57BL/6 background [10,11]. In the first experiment, all animals were allowed free access to food. They were assigned to four groups: a control group ($n=6$) receiving a single intraperitoneal injection of the vehicle used for fenofibrate administration; a treated group ($n=6$) receiving a single intraperitoneal injection of fenofibrate (300 mg/kg); two groups were constituted in the PPARα−/− mice ($n=6$ in each group). As with the control mice, the first group received only the vehicle, whereas the second received the fenofibrate treatment previously described. In a second experiment, using a similar procedure, six control and six PPARα−/− mice were allowed free access to food and were killed 3 h following the beginning of the dark, and the remaining two groups

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were starved for 30 h. At this stage, all animals were killed between 9 and 11 a.m. and the livers were collected and immediately frozen in liquid nitrogen.

2.2. Mitochondria preparations and enzymatic activities

Liver mitochondria were prepared by differential centrifugation according to Wrutniak et al. [16]. Citrate synthase, cytochrome *c* oxidase (COX) and succinate ubiquinone oxidoreductase (complex II) activities were measured according to Rochard et al. [26].

2.3. Western blot analysis

Mitochondrial proteins (50 μg) were electrophoresed through SDS–PAGE gel, transferred to a PDVF membrane and detected by a chemiluminescent Western blot procedure. Anti-RHTII, anti-PPARγ2 and anti-mt-TFA antisera have been described previously [16,17,25]. Anti-E2-PDH antiserum was kindly provided by Dr C. Marsac (INSERM, Paris). Quantification of signal intensities was carried out with a PhosphorImager (Molecular Dynamics) and normalized against E2-PDH used as invariant.

3. Results

3.1. Fenofibrate treatment or fasting specifically increased COX activity

To test the influence of fenofibrate or fasting upon mitochondrial activity, we measured several enzymatic activities: two enzymes encoded by nuclear genes, citrate synthase (a key enzyme of the tricarboxylic acid cycle) and succinate ubiquinone oxidoreductase (complex II), and one enzymatic complex encoded by both nuclear and mitochondrial genes, COX.

In control or PPARα−/− mice, fenofibrate did not influence citrate synthase and complex II activities (Table 1). On the other hand, it induced a significant rise in COX activity in control animals as well as in mice lacking PPARα (Table 1). Similar changes in COX activities were induced by fasting in control or PPARα-deficient mice (Table 1). In addition, as described for fenofibrate, fasting did not influence citrate synthase or complex II activities (Table 1).

3.2. Fenofibrate treatment or fasting increased p43 and mt-PPAR mitochondrial levels

The observation that, in contrast to citrate synthase or complex II, COX activity is up-regulated by fenofibrate or fasting suggests a major influence of these treatments on mitochondrial genome expression. To test this possibility, we studied their influence on the mitochondrial amounts of mt-TFA, a constitutive transcription factor of the organelle, and p43, a T3-dependent mitochondrial transcription factor. The amounts of the mitochondrial PPARγ2-related protein, mt-PPAR [25], were also monitored.

In comparison to control mice, fenofibrate induced an up to two-fold increase in the levels of p43 or mt-PPAR (Fig. 1). In addition, whereas the induction of mt-PPAR by fenofibrate was completely abolished in mice lacking PPARα, the rise in amounts of p43 was not altered in these animals (Fig. 1). Similar results were obtained for the influence of fasting (Fig. 2). However, the mitochondrial levels of mt-TFA were not altered by fenofibrate, fasting or in PPARα gene invalidation (Fig. 3).

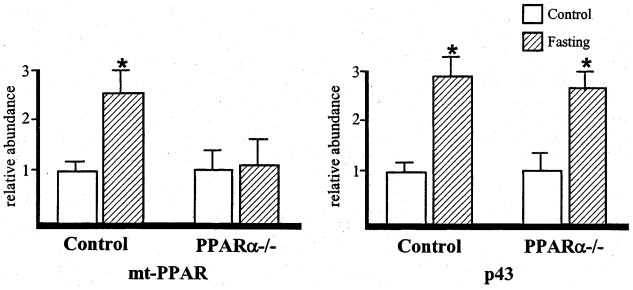

Fig. 2. Fasting increased the mitochondrial levels of p43 and mt-PPAR. Data are derived from Western blot analysis of mitochondrial proteins (50 μg) using rat liver mitochondrial extracts and anti-RHTII, anti-PPARγ2 or anti-E2-PDH antisera. Quantification of signal intensities was carried out with a PhosphorImager (Molecular Dynamics) and normalized against the levels of the mitochondrial protein E2-PDH. Data are expressed as the mean of results obtained in four animals. $*P < 0.01$ relative to control.

4. Discussion

In the present study, we found that fenofibrate or fasting do not influence citrate synthase or complex II activity. On the other hand, these treatments induced a significant rise in COX activity. Such a difference could reflect the genetic origin of these enzymes. Whereas the latter are encoded by nuclear genes, COX is a multimeric complex involving distinct subunits encoded by nuclear genes and by the mitochondrial genome. Therefore, specific regulation of COX activity by fenofibrate or fasting suggests that the two treatments affect

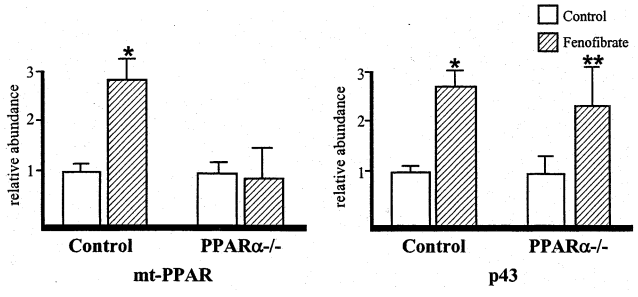

Fig. 1. Fenofibrate treatment increased the mitochondrial levels of p43 and mt-PPAR. Data are derived from Western blot analysis of mitochondrial proteins (50 μg) using rat liver mitochondrial extracts and anti-RHTII, anti-PPARγ2 or anti-E2-PDH antisera. Quantification of signal intensities was carried out with a PhosphorImager (Molecular Dynamics) and normalized against the levels of the mitochondrial protein E2-PDH. Data are expressed as the mean of results obtained in four animals. $*P < 0.01$; $**P < 0.05$ relative to control.

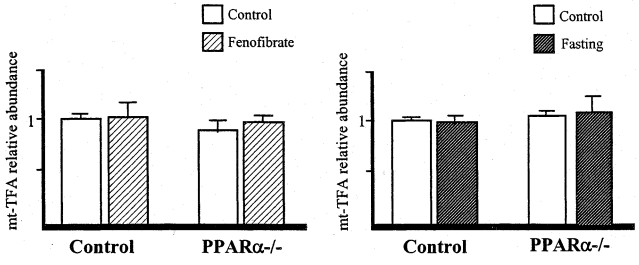

Fig. 3. Mitochondrial levels of mt-TFA are not altered by fenofibrate or fasting. Data are derived from Western blot analysis of mitochondrial proteins (50 μg) using rat liver mitochondrial extracts and anti-mt-TFA antiserum. Mt-TFA synthesized in rabbit reticulocyte was used as control. Quantification of signal intensities was carried out with a PhosphorImager (Molecular Dynamics) and normalized against the levels of the mitochondrial proteins E2-PDH. Data are expressed as the mean of results obtained in four animals.

Table 1
Fenofibrate treatment or fasting increased COX activity but not citrate synthase or complex II activities

	Control	Control fenofibrate	PPARα−/−	PPARα−/− fenofibrate	Control	Control fasting	PPARα−/−	PPARα−/− fasting
Citrate synthase	100 ± 2	121 ± 4	99 ± 5	122 ± 3	100 ± 8	121 ± 7	105 ± 13	109 ± 6
Complex II	100 ± 3	96 ± 2	102 ± 5	99 ± 3	100 ± 11	112 ± 11	121 ± 13	130 ± 9
COX	100 ± 1	175 ± 5**	97 ± 7	177 ± 11*	100 ± 4	169 ± 5••	96 ± 8	164 ± 12•

Mitochondrial enzyme activities are expressed as a percentage of the values obtained in C57BL/6 control mice. Data are the mean of duplicate determinations of six samples/group (± S.E.M.). $^{**}P < 0.001$ relative to control (in the absence of fenofibrate); $^{*}P < 0.005$ relative to PPARα−/− (in the absence of fenofibrate); $^{••}P < 0.001$ relative to control (ad libitum); $^{•}P < 0.005$ relative to PPARα−/− (ad libitum).

mitochondrial activity essentially by stimulating mitochondrial genome transcription, as already described [18].

In previous studies, we demonstrated the occurrence of truncated forms of members of the nuclear receptor family in the mitochondrial matrix [16,25]. We have also established that p43, a T3-binding protein synthesized by the use of an internal AUG occurring in the c-erb Aα1 mRNA [27] acts as a T3-dependent transcription factor of the mitochondrial genome [17]. In addition, we demonstrated [25] that it binds to the mitochondrial D-loop by forming a complex including mt-PPAR, a PPARγ2-related protein devoid of the carboxy-terminus of the nuclear receptor.

Interestingly, we report here that stimulation of organite activity by fenofibrate or fasting is related to an increase in mitochondrial amounts of p43. As we have shown that p43 overexpression stimulates mitochondrial genome transcription [17] and COX activity [16], but not citrate synthase activity, we suggest that such a mechanism is involved in the influence of the two treatments at mitochondrial level. This possibility is concordant with the present observation that regulation of p43 expression is not abolished in PPARα-deficient mice, and that stimulation of COX activity is not grossly impaired in these animals. In addition, the observation that mt-TFA is not influenced by fenofibrate or fasting suggests that this constitutive mitochondrial transcription factor is not involved in the regulation of mitochondrial activity.

Another striking result is the observation that, as previously reported for PPARγ nuclear receptors [28], mt-PPAR amounts are increased by a fenofibrate treatment. This is concordant with the stimulation of mt-PPAR abundance by clofibrate in rat liver mitochondria already documented [25]. In contrast to p43, this regulation is abrogated in PPARα-deficient mice, thus suggesting that this nuclear receptor plays a key role in the regulation of mt-PPAR expression. However, despite the lack of influence of fibrate or fasting on mt-PPAR in PPARα-deficient mice, stimulation of COX activity is maintained in knocked-out animals in these conditions.

Our data indicate that the rise in COX activity and mitochondrial amounts of p43 induced by fenofibrate also occurred in PPARα−/− mice. This is probably an interesting result clearly establishing that not all fibrate influences are mediated by the nuclear receptor PPARα.

In conclusion, this study clearly suggests that fibrates and fasting influence mitochondrial activity, and that this action occurs essentially at the level of the mitochondrial genome. Moreover, their influence on the amounts of mitochondrial regulators, such as p43 and mt-PPAR, is probably involved in this regulation. In addition to a recent paper demonstrating that there is a low reserve of COX activity in vivo [29], this observation suggests that the in vivo control of respiration by COX activity could be dependent on mitochondrial genome encoded subunits.

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