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Modification of respiratory-chain enzyme activities in brown adipose tissue mitochondria by idebenone (hydroxydecyl-ubiquinone)

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Abstract Idebenone (IDE), a synthetic analog of coenzyme Q, strongly activates glycerol phosphate (GP) oxidation in brown adipose tissue mitochondria. GP oxidase, GP cytochrome *c* oxidoreductase and GP dehydrogenase activities were all significantly stimulated by 13 μM IDE. Substituted derivatives of IDE acetyl- and methoxyidebenone had similar activating effects. When succinate was used as substrate, no activation by IDE could be observed. The activation effect of IDE could be explained as release of the inhibition of glycerol phosphate dehydrogenase by endogenous free fatty acids. NADH oxidoreductase activity and oxidation of NADH-dependent substrates were inhibited by IDE. The extent of the inhibition and IDE concentration dependence varied when various substrates were tested, being highest for pyruvate and lowest for 2-oxoglutarate. This study thus showed that the effect of IDE on various mitochondrial enzymes is very different and thus its therapeutic use should take into account its specific effect on various mitochondrial dehydrogenases in relation to particular defects of mitochondrial respiratory chain.

Keywords Idebenone · NADH · Glycerol phosphate and succinate oxidoreductase activities · Brown adipose tissue mitochondria

Abbreviations

CoQ	coenzyme Q
IDE	idebenone (hydroxydecyl-ubiquinone)
ROS	reactive oxygen species
GP	glycerol-3-phosphate
mGPDH	mitochondrial glycerol-3-phosphate dehydrogenase
BSA	bovine serum albumin
DCIP	2,6-dichlorophenol indophenol

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Introduction

Coenzyme Q (CoQ), the only non-protein molecule of the mitochondrial energy provision system, plays an important role as a mobile electron carrier in the respiratory chain function. As compared with the other respiratory-chain components, the molecular ratio of CoQ is in large excess (Kröger and Klingenberg 1973). The CoQ content is decreased in ageing and in a number of diseases (Dhanasekaran and Ren 2005; Linnane and Eastwood 2006; Sohal and Forster 2007), of which mitochondrial oxidative phosphorylation disorders represent genetically impaired CoQ synthesis (Quinzii et al. 2007).

CoQ supplementation showed a clinical improvement and corrected biochemical disarrangements. Idebenone

(IDE), a synthetic analog of CoQ (with a 10-carbon side chain ending with a hydroxyl group) is also available for the therapy of mitochondrial disorders (Zs-Nagy 1990). It can be used either as a substitutive electron carrier or an antioxidant compound similarly as natural CoQ (Geromel et al. 2002; Rustin et al. 2004). It has the advantage of lower hydrophobicity in comparison with natural long-chain CoQ. However, there are accumulating data showing that analogs of CoQ cannot fully replace endogenous CoQ because they behave differently when reacting with various dehydrogenases (Brière et al. 2004; Degli Esposti et al. 1996). It was also suggested that short-chain CoQ analogs including IDE, enhance superoxide formation by the complex I (Genova et al. 2003). Moreover, the antioxidative capacity of CoQ and its analogs is highly dependent on the topology of reactive oxygen species (ROS) generation in the respiratory chain (James et al. 2005; Turrens 2003). To understand all the mechanisms of the beneficial effects of CoQ analogs as additional potential therapeutic agents it is necessary to obtain more information about their interaction with various mitochondrial dehydrogenases and also about their ability to substitute for CoQ in various reactions in which CoQ is involved.

In our previous experiments we found that brown adipose tissue mitochondrial glycerol phosphate dehydrogenase (mGPDH) represents a new site of the respiratory chain where reactive oxygen species are generated (Drahota et al. 2002, 2003; Rauchová et al. 2001; Vrbacký et al. 2007). In brown adipose tissue mitochondria where mGPDH is highly expressed and has comparable activity with succinate dehydrogenase we detected that IDE strongly depressed both glycerol phosphate (GP)- and succinate-dependent ROS production (Rauchová et al. 2006) similarly as CoQ_3 (Drahota et al. 2002).

We have also observed that GP oxidation can be increased by CoQ_3 (Rauchová et al. 1992, 2003). However this activation was specific only for GP and could not be detected when succinate was used as substrate. Therefore we tested whether this specific activation of GP-dependent oxidation can be induced by IDE. We also measured the effect of IDE on oxidation of various NADH-dependent substrates in intact brown adipose tissue mitochondria, because it has been shown that IDE is a strong inhibitor of NADH/CoQ reductase in beef heart submitochondrial particles (Degli Esposti et al. 1996).

Materials and methods

Isolation of brown adipose tissue mitochondria

The experiments were performed on brown adipose tissue mitochondria from adult male hamsters (*Mesocricetus auratus*) adapted at 4 °C for 3 weeks and mitochondria were isolated as described by Cannon and Lindberg (1979). The isolation medium was 250 mM sucrose, 10 mM Tris–HCl, and 1 mM EDTA, pH 7.4. For experiments freshly isolated or frozen-thawed mitochondria stored at −80 °C were used.

Determination of enzyme activities

Enzyme activities were determined spectrophotometrically at room temperature as cytochrome *c* oxidoreductase following the cytochrome *c* reduction at 550 nm, as 2, 6-dichlorophenol indophenol (DCIP) oxidoreductase following DCIP reduction at 600 nm or ferricyanide oxidoreductase following ferricyanide reduction at 420 nm in 1 ml of medium containing 50 mM KCl, 20 mM Tris–HCl, 1 mM EDTA and 2 mM KCN, pH 7.4, using mitochondrial protein 0.050–0.075 mg/ml. Concentration of electron acceptors was 0.1, 0.1 and 0.5 mM for cytochrome *c*, DCIP and ferricyanide, respectively. The reaction was started by addition of 25 mM GP, 25 mM succinate or 0.075 mM NADH. Extinction coefficients 19, 21, and 1 $mmol^{-1}$ cm^{-1} for cytochrome *c*, DCIP and ferricyanide were used, respectively.

GP:CoQ oxidoreductase activities were measured by following the decrease of absorbance of the oxidized quinones upon reduction at the 275 nm in 1 ml of medium containing 50 mM KCl, 20 mM Tris–HCl, 1 mM EDTA and 2 mM KCN, pH 7.4, using mitochondrial protein 0.050–0.075 mg/ml. Concentrations of CoQ_1, CoQ_2, CoQ_3 and IDE were 150, 75, 40 and 60 μM, respectively. The reaction was started by addition of 25 mM GP. For short-chain CoQ homologs (CoQ_1, CoQ_2 and CoQ_3) and IDE the extinction coefficient 13.6 $mmol^{-1}$ cm^{-1} was used.

Mitochondrial oxygen consumption

Oxygen consumption by mitochondria was measured at 30 °C with a High Resolution Oxygraph (Oroboros, Austria) in 2 ml of incubation medium containing 100 mM KCl, 10 mM Tris–HCl, 5 mM K-phosphate, 3 mM $MgCl_2$ and 1 mM EDTA, pH 7.4, using 0.15–0.30 mg of mitochondrial protein/ml. Glycerol phosphate (10 mM), succinate (10 mM), pyruvate (10 mM) + malate (2.5 mM), palmitoyl carnitine (0.0025 mM) + malate (2.5 mM), β-hydroxy butyrate (10 mM) and 2-oxoglutarate (10 mM) + malate (2.5 mM) were added to start the reaction. The oxygraphic curves presented are the first negative derivatives of oxygen tension changes. For calculation of oxygen uptake rates and for presentation of oxygraphic curves OROBOROS software Datlab 3 was used (Gnaiger et al. 1995). Oxygen uptake is expressed as pmol oxygen/s/mg mitochondrial protein.

Protein estimation

Proteins were determined according to Lowry et al. (1951) using bovine serum albumin as standard.

Chemicals

IDE was kindly provided by Takeda (Osaka, Japan) and CoQ_3 by Eisai Co. (Tokyo, Japan). Methoxyidebenone and acetylidebenone were kind gifts from Prof. P. Bernardi, Padova, Italy.

CoQ_1, CoQ_2, tris(hydroxymethyl) aminomethane, cytochrome *c* (horse heart type III), glycerol-3-phosphate, succinate, and NADH were purchased from Sigma (USA). Other chemicals were of the highest purity commercially available.

Results

Idebenone and glycerol phosphate-, succinate- and NADH-dependent oxidoreductase activities

We have evaluated to which extent IDE can activate the GP branch of the respiratory chain. Using frozen-thawed brown adipose tissue mitochondria we compared the effect of IDE on GP-, succinate- and NADH oxidoreductase activities. Data summarized in Table 1 show that GP:cytochrome *c* oxidoreductase and GP:DCIP oxidoreductase activities were increased by 13 μM IDE about two-fold. However, at the same concentration of IDE no activating effect on succinate oxidoreductases could be detected. Activities of NADH:cytochrome *c* oxidoreductase and NADH:DCIP oxidoreductase were inhibited by IDE. The inhibition of NADH:cytochrome *c* oxidoreductase was more pronounced than that of NADH:DCIP oxidoreductase. All these data thus showed a quite different effect of IDE on three branches of the respiratory chain tested.

Activities of ferricyanide oxidoreductases for all substrates tested (GP-, succinate- and NADH) were higher when compared with cytochrome *c* and DCIP oxidoreductases and were not changed by added IDE (Table 1).

Table 1 The effect of idebenone on glycerol phosphate, succinate and NADH oxidoreductase activities

	Control (nmol min^{-1} mg $protein^{-1}$)	Idebenone (nmol min^{-1} mg $protein^{-1}$)	Percent
Cytochrome *c* oxidoreductase			
Glycerol phosphate	272.4±6.6	566.8±19.2	225 ($p \leq 0.001$)
Succinate	180.7±15.3	196.9±8.6	109 (n.s.)
NADH	353.2±28.5	117.8±10.9	33 ($p \leq 0.01$)
DCIP oxidoreductase			
Glycerol phosphate	111.7±4.5	243.1±2.9	219 ($p \leq 0.001$)
Succinate	85.6±6.4	95.9±10.7	112 (n.s.)
NADH	108.5±1.5	80.0±1.9	75 ($p \leq 0.01$)
Ferricyanide oxidoreductase			
Glycerol phosphate	1,045.7±202.4	1,011.5±243.2	97 (n.s.)
Succinate	506.3±81.2	493.3±65.9	98 (n.s.)
NADH	1,574.6±158.9	1,591.4±139.9	104 (n.s.)

Frozen-thawed mitochondria were used; added idebenone was 13 μM. Enzyme assays were described in "Materials and methods". Data presented are means±SEM from three to four experiments.

Table 2 Idebenone and short-chain CoQ homologs as electron acceptors for glycerol phosphate oxidoreductase activities

Acceptor	Specific activity (nmol min^{-1} mg $protein^{-1}$)
Glycerol phosphate cytochrome *c* oxidoreductase	
Cytochrome *c*	234.6±22.6
Glycerol phosphate CoQ oxidoreductases	
Idebenone	400.8±4.5
Coenzyme Q_1	406.0±6.9
Coenzyme Q_2	262.5±4.9
Coenzyme Q_3	199.3±13.0

Frozen-thawed mitochondria were used; enzyme assays were described in "Materials and methods". Data presented are means±SEM from four to five experiments.

Idebenone as an electron acceptor for glycerol phosphate-dependent oxidoreductase activity

In our previous paper we showed that the most suitable electron acceptor for GP:CoQ oxidoreductase activity in brown adipose tissue mitochondria was the homolog CoQ_1 (Rauchová et al. 1997), in agreement with the activities of succinate:CoQ and NADH:CoQ oxidoreductase (Estornell et al. 1993; Fato et al. 1996, Lenaz et al. 1995). In this study we estimated this enzyme activity with IDE as the electron acceptor and found that the GP:IDE oxidoreductase activity was comparable with that reached with CoQ_1 and higher than that obtained with CoQ_2 and CoQ_3 (Table 2).

The effect of idebenone derivatives on glycerol phosphate oxidoreductase activities

In further experiments we compared the effect of IDE on GP:DCIP oxidoreductase activity together with the effect of its two derivatives, methoxy-and acetylidebenone. We found an activating effect for all three compounds with similar K_m in the range of about 15 μM (Fig. 1a–c).

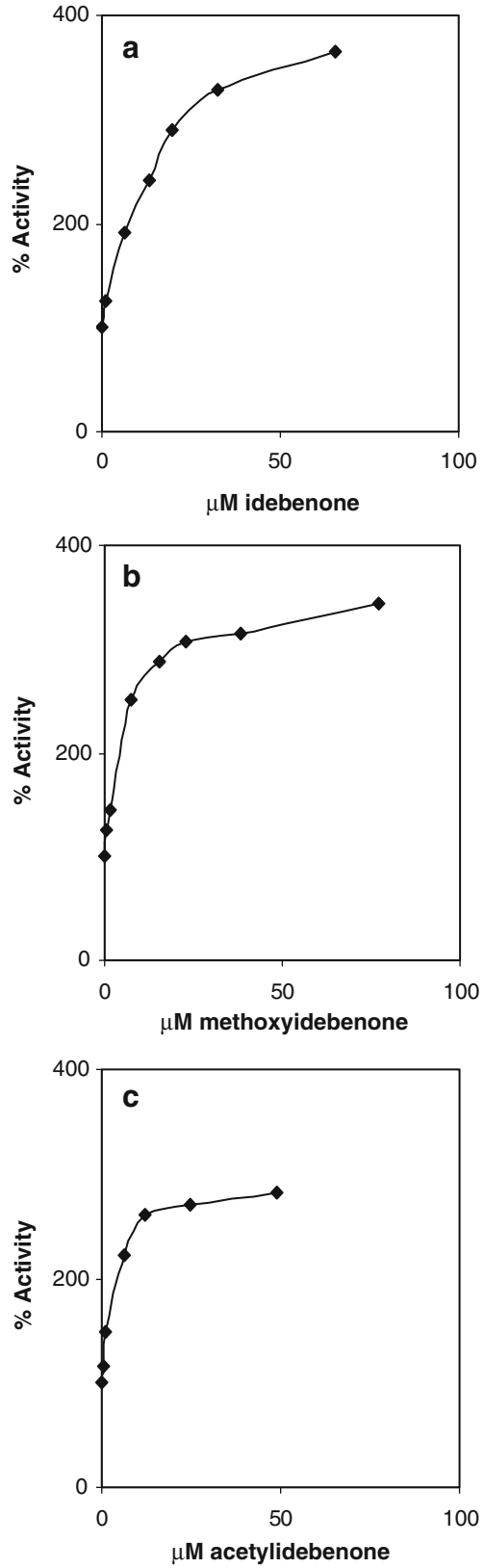

Fig. 1 The effect of idebenone (**a**), methoxyidebenone (**b**) and acetylidebenone (**c**) on glycerol phosphate DCIP oxidoreductase activity

Activation by acetylidebenone was lower than that induced by IDE or methoxyidebenone. Similar effects were found for GP:cytochrome *c* oxidoreductase (not shown).

Idebenone and glycerol phosphate- and succinate-dependent respiration

The activating effect of IDE was also observed when the rate of GP-dependent oxygen uptake was determined using freshly isolated brown adipose tissue mitochondria. Maximum activation of the GP oxidation was found at 15–20 μM IDE (Fig. 2a,b). A sharp inhibition in oxygen

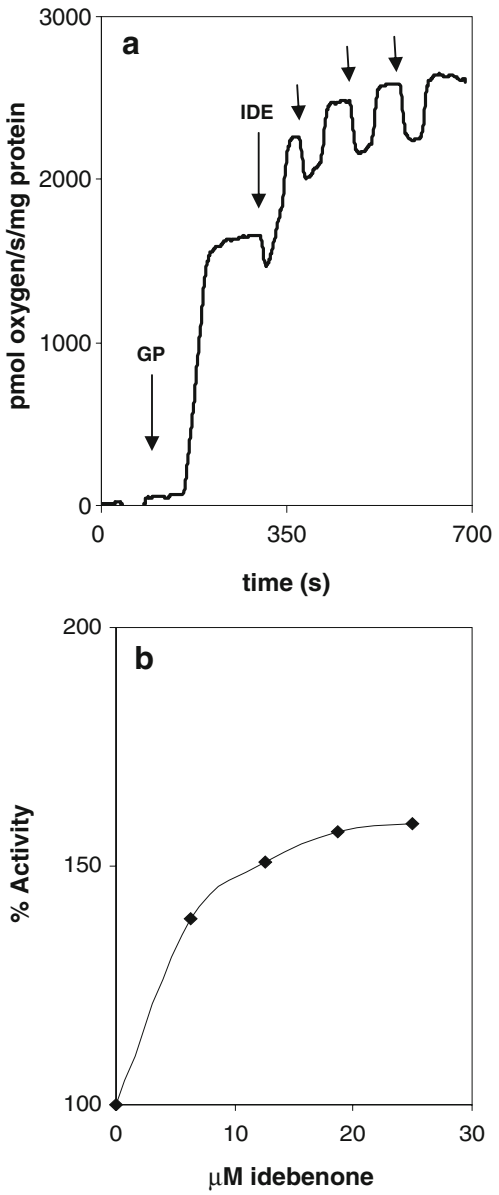

Fig. 2 **a**, **b** The activation of glycerol phosphate oxidation by idebenone. **a** As indicated, 10 mM glycerol phosphate (*GP*) and four additions of 13 μM idebenone (*IDE*) were added, **b** % activation of the GP-dependent oxidation by added IDE

consumption after addition of IDE is due to addition of ethanol solution. In parallel control experiments we saw that the sharp decrease of the oxygen consumption rate quickly returned to original values after addition of pure ethanol (not shown). Similarly as in the previous experiments (Table 1), the activating effect of IDE was more pronounced when GP was oxidized, in comparison with its effect on succinate oxidation (Fig. 3a,b). In some preparations an activation effect on succinate oxidation could also be detected but this activation was not statistically significant. The average value of the IDE activation effect for GP oxidation was 163% (Table 3), which was lower in comparison with IDE activation of GP:cytochrome *c* oxidoreductase activity (Table 1).

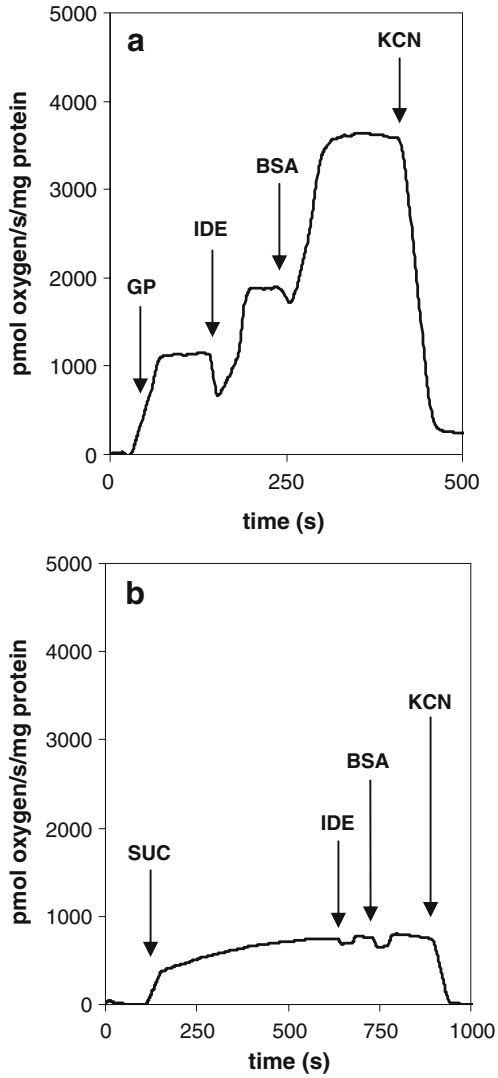

Fig. 3 **a**, **b** The effect of idebenone on glycerol phosphate- and succinate-dependent oxygen consumption. **a** As indicated, 10 mM glycerol phosphate (*GP*), 13 μM idebenone (*IDE*) 0.5 mg BSA/ml (*BSA*) and 25 mM KCN (*KCN*) were added. **b** As indicated, 10 mM succinate (*SUC*), 13 μM idebenone (*IDE*) 0.5 mg BSA/ml (*BSA*) and 25 mM KCN (*KCN*) were added

Table 3 The activating effect of idebenone on the rate of glycerol phosphate- or succinate-dependent oxygen uptake of freshly isolated brown adipose tissue mitochondria

Substrate	Control	+Idebenone	Activation (%)
GP (n=10)	777.4±66.3	1,265.9±117.5	163 ($p \leq 0.01$)
SUC (n=8)	357.4±39.4	437.6±48.8	122 (n.s.)

Mitochondrial protein was 0.15–0.30 mg/ml, glycerol phosphate (GP) and succinate (SUC) were 10 mM, idebenone 13 μM. Data presented are means±SEM, *n* indicates the number of experiments.

The effect of BSA, oleate and cytochrome *c* on glycerol phosphate-dependent respiration

When oleate was added to intact mitochondria, the GP oxidation was strongly inhibited. This inhibition could be partially released by IDE (Fig. 4). The rate of GP oxidation (without or with oleate) activated by IDE could be further increased by the addition of BSA (Fig. 3a and 4). In the presence of BSA no activating effect of IDE could be detected (not shown).

Activation by IDE could also be observed in frozen-thawed mitochondria. Under these experimental conditions, however, the rate of GP oxidation was lower due to the release of cytochrome *c* from disrupted mitochondria. Addition of cytochrome *c* and BSA increased the respiratory rate to values similar to those observed in intact mitochondria. Under these experimental conditions, in the presence of cytochrome *c* and BSA, IDE had no activating effect (Fig. 5). Altogether these findings indicate that the activation potential of IDE is lower than that of BSA.

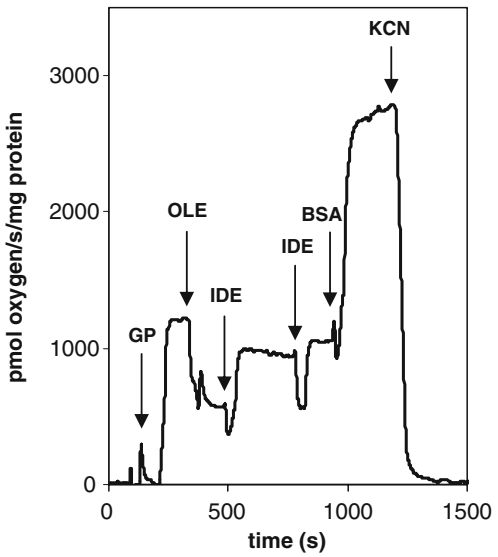

Fig. 4 Inhibition of added oleate released by idebenone and serum albumin. As indicated 10 mM glycerol phosphate (*GP*), 12.5 μM oleate (*OLE*), twice 13 μM idebenone (*IDE*), 0.5 mg BSA/ml (*BSA*) and 25 mM KCN (*KCN*) were added

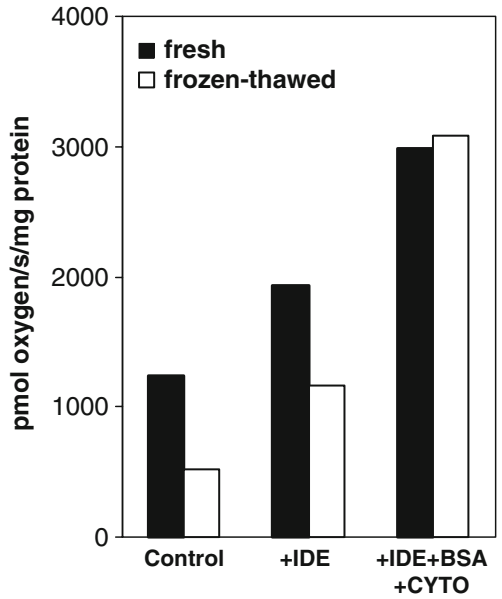

Fig. 5 The activation effect of idebenone, serum albumin and cytochrome *c* in freshly isolated and frozen-thawed mitochondria. Control contains mitochondria with 10 mM glycerol phosphate; as indicated, 13 μM idebenone (+*IDE*), 0.5 mg BSA/ml (+*BSA*) and 20 μM cytochrome *c* (+*CYTO*) were added

Idebenone and oxidation of NADH-dependent substrates

We found a strong inhibitory effect of IDE on the rate of pyruvate + malate oxidation (Fig. 6a). The rate of oxygen uptake was inhibited by 13 μM IDE to 31% and by 26 μM to 14% of the original rate. The inhibition could be reversed by added BSA. Addition of 1 mg BSA/ml increased the respiration rate to 44% and addition of 2 mg BSA/ml to 81% of the original value (Fig. 6a).

Oxidation of palmitoyl carnitine + malate was also strongly depressed by increasing concentration of IDE. At 13 μM IDE the respiration rate was decreased to 41%, at 26 μM to 28% and at 39 μM to 24% of the original rate (Fig. 6b). The total amount of oxygen consumed after addition of palmitoyl carnitine (2.5 μM) remained the same at increasing IDE concentrations (102, 95 and 103% of original values, respectively). This indicates that in the presence of IDE added palmitoyl carnitine can be completely oxidized but at a lower rate.

The β-hydroxy butyrate (Fig. 6c) and 2-oxoglutarate + malate (Fig. 6d) oxidation was also inhibited by IDE but in comparison with pyruvate + malate oxidation, to a lower extent, and a higher concentration of IDE was required for maximum inhibition. The rate of β-hydroxy butyrate oxidation was decreased by 39 μM IDE to 66% of the original values and that of 2-oxoglutarate was decreased by 78 μM IDE to 53%. When the inhibitory effect of two concentrations of IDE (32 and 160 μM) was tested quick inhibition after addition of IDE was observed. The inhibition of the respiration rate was higher at higher IDE concentration and the residual rate was stable during 10 min incubation period.

Discussion

CoQ has an important role in the mitochondrial respiratory chain function as an acceptor of reducing equivalents from various mitochondrial dehydrogenases (Lenaz et al. 2007). Besides this well-known function there is growing evidence for its new roles in cellular metabolism (Turunen et al. 2004). Nowadays CoQ and IDE are the only two therapeutic agents used. It would be profitable to obtain other CoQ analogs to develop further pharmacological drugs. However, their mechanisms of beneficial action and possible interactions with various branches of the respiratory chain should be fully clarified because experimental data showed important variations of CoQ analogs in their interactions (Degli Esposti et al. 1996). Some disturbing effects on the function of complex I were also observed (Degli Esposti 1998; Genova et al. 2003). Therefore, it is necessary to obtain more information about the interactions of particular CoQ analogs.

We measured the effect of IDE on the oxidation rates of two flavoprotein-dependent substrates, GP and succinate, three NADH-dependent substrates, pyruvate, 2-oxoglutarate, β-hydroxybutyrate and on the oxidation rate of palmitoyl carnitine.

Our data on frozen-thawed brown adipose tissue mitochondria showed that IDE increases GP oxidoreductase activities when cytochrome *c* or DCIP are used as electron acceptors. The activation was specific for GP and could not be obtained when succinate was used as substrate. Similar results were obtained when GP and succinate oxidation was measured using intact brown adipose tissue mitochondria. Because a similar activating effect as IDE was found with the acetyl- and methoxyidebenone, chemical modification of IDE molecule had evidently no dramatic effect on the activating capacity. The activating effect of IDE could not be explained as a direct transfer of electrons from mGPDH flavoprotein to cytochrome *c* bypassing complex III, since we found that GP:cytochrome *c* oxidoreductase was antimycin A sensitive. Oxygraphic measurements also showed that GP-dependent oxidation activated by IDE was inhibited by antimycin A. The residual rate, about 15% of original values could be partially inhibited by KCN, which indicates that the potential bypass of electrons to cytochrome *c* oxidase can not be higher than 5% of the total flux (unpublished data).

On the basis of our previous experiments (Rauchová and Drahota 1984; Rauchová et al. 2003) we could explain the specific increase of GP oxidation as the release of

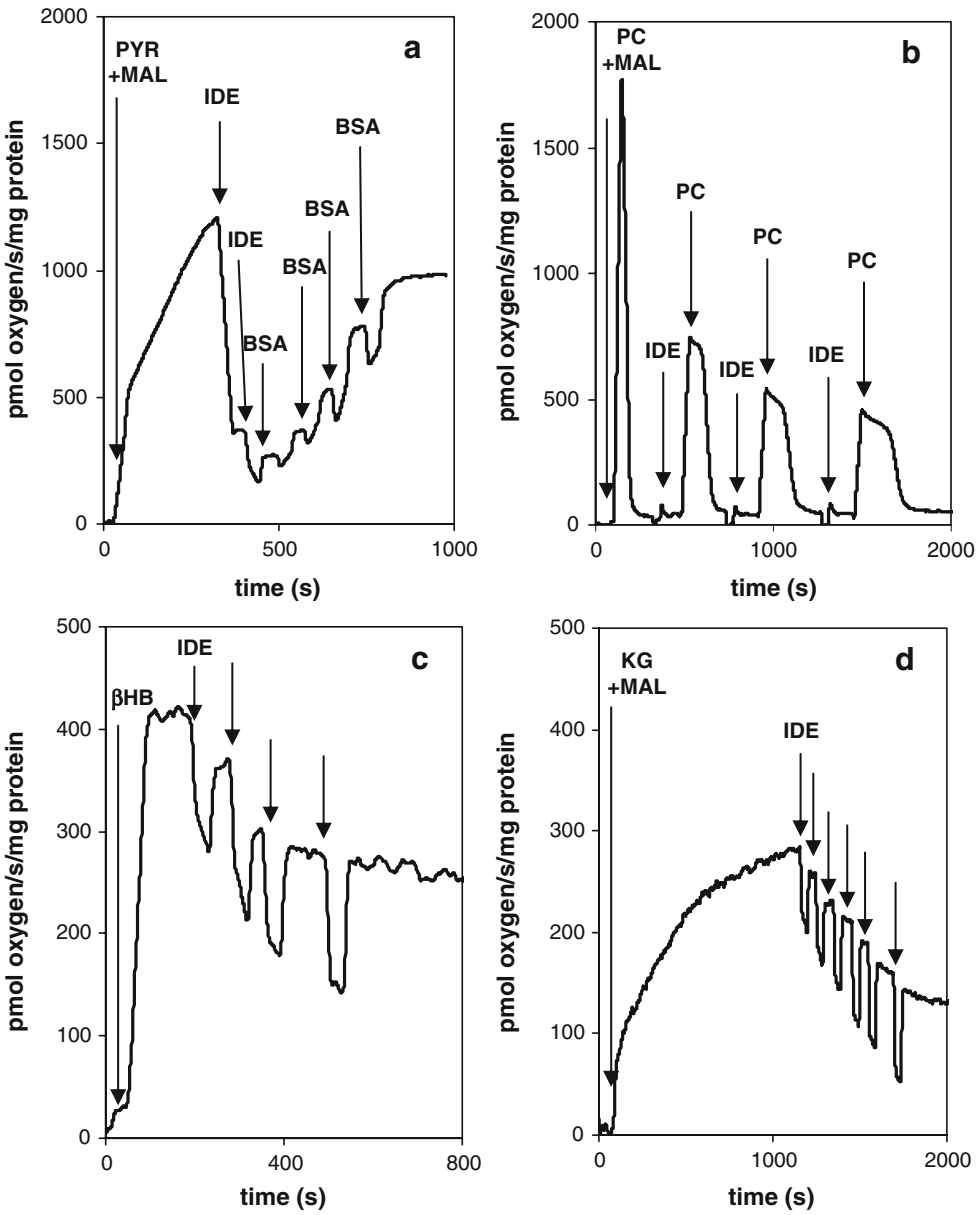

Fig. 6 a–d The inhibition of NADH-dependent substrates by idebenone. **a** As indicated, 10 mM pyruvate and 2.5 μM malate (*PYR+MAL*), two additions of 13 μM idebenone (*IDE*) and 0.5 mg BSA/ml (*BSA*) were added. **b** As indicated, 2.5 μM palmitoyl carnitine and 2.5 μM malate (*PC+MAL*), three times 13 μM idebenone (*IDE*) were added. **c** As indicated 10 mM β-hydroxy butyrate (*βHB*) and four times 13 μM idebenone (*IDE*) were added. **d** As indicated, 10 mM 2-oxoglutarate and 2.5 μM malate (*KG+MAL*) and six times of 13 μM idebenone (*IDE*) were added

inhibitory action of endogenous free fatty acids on mGPDH. IDE thus activates the transfer of electrons from the enzyme to the CoQ pool inhibited by free fatty acids because a similar effect can be obtained by their extraction by BSA (Houštěk and Drahota 1975). However, activation by BSA was higher than that by IDE and in the presence of BSA IDE had no activating effect. Evidently the two substances activate the enzyme through different mechanisms. IDE can compete for fatty acid binding sites or overpass the fatty acid block that was localized between mGPDH and the CoQ pool (Rauchová and Drahota 1984) and transport electrons from mGPDH to CoQ. Both these mechanisms are evidently less efficient than the complete extraction of free fatty acids by high-affinity binding sites on the BSA molecule.

The fact that free fatty acids are involved in IDE activation is also supported by measurements of succinate oxidation. Neither succinate oxidoreductase nor succinate oxidase activities are increased by IDE. This is in agreement with our previous observations that those activities are not inhibited by free fatty acids (Houštěk and Drahota 1975). Altogether our data showed that IDE, besides its involvement in various metabolic reactions, has another specific function in releasing the inhibitory effect induced by free fatty acids on mGPDH. The free fatty acids inhibitory effect is presumably the result of their competitive interaction with the natural CoQ binding site on the enzyme (Rauchová et al. 2003).

Using intact brown adipose tissue mitochondria we tried to extend the data describing in beef heart submitochondrial

particles the inhibitory effect of IDE on complex I activity (Degli Esposti et al. 1996). We tested NADH oxidoreductase activities and the rate of oxygen consumption with various NADH-dependent substrates. We observed a strong inhibition of NADH oxidoreductase by IDE to 33% of original activity when cytochrome *c* was used as electron acceptor. When DCIP was used the inhibition was lower, only to 75%. The inhibitory effect of IDE is therefore exerted on the CoQ binding site, which partially contributes to DCIP reduction and is located downstream the ferricyanide and non the physiological DCIP reducing site.

When we tested the inhibitory effect of IDE on respiration of NADH-dependent substrates on intact mitochondria, we found the highest effect on pyruvate + malate oxidation. The inhibition was completely reversed by BSA evidently due to IDE binding by BSA.

The respiration with other respiratory substrates: palmitoyl carnitine, 2-oxoglutarate and β-hydroxy butyrate was inhibited to a lower extent and higher concentrations of IDE were required to reach maximum inhibition.

Inhibition of complex I by IDE was explained by modified stoichiometry between NADH oxidation and quinone reduction. Under these conditions reduction of the CoQ analog was shifted from two electrons to an incomplete reaction that resulted in an increase of unstable intermediates acting as prooxidants and including generation of ROS which could affect the Fe-S cofactors of complex I (Degli Esposti 1998; Unemoto and Hayashi 1989). However, differences in IDE inhibitory effects between pyruvate, 2-oxoglutarate, β-hydroxy butyrate and palmitoyl carnitine indicate that, in addition to the inhibition of complex I, other inhibitory mechanisms could be involved. For example, it was found that CoQ_o analog forms an adduct with dihydrolipoamide present in pyruvate and 2-oxoglutarate dehydrogenases, which could explain its cellular toxicity. This interaction was described as covalent binding but it was also found that CoQ_1 and CoQ_2, which do not react with lipoamide, may prevent CoQ_o binding to 2-oxoglutarate dehydrogenase (MacDonald et al. 2004).

We may thus conclude that our data extended our knowledge about the interaction of IDE with mGPDH and about its ability to release the inhibitory effect of free fatty acids on this enzyme. The data suggest that the inhibitory effect is exerted by occupying the CoQ reducing site of the enzyme, thus preventing transfer of reducing equivalents to the CoQ pool and complex III. Nevertheless, it is also possible that IDE can react directly with the enzyme-reduced flavine and bypass the fatty acid inhibition. Data related to the inhibition of NADH-dependent substrate oxidation by IDE show that there is not a common mechanism responsible for IDE action on various branches of the mitochondrial respiratory chain: IDE may activate, inhibit or have no effect. This study suggests that any therapeutic use of CoQ analogs should consider their specific effect on each mitochondrial dehydrogenase in relation to the particular defects of the respiratory chain.

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References

Brière JJ, Schlemmer D, Chrétien D, Rustin P (2004) Biochem Biophys Res Commun 316:1138–1142
Cannon B, Lindberg O (1979) Methods Enzymol 55:65–78
Degli Esposti M (1998) Biochim Biophys Acta 1364:222–235
Degli Esposti M, Ngo A, Ghelli A, Benelli B, Carelli V, McLennan H, Linnane AW (1996) Arch Biochem Biophys 330:395–400
Dhanasekaran M, Ren J (2005) Curr Neurovasc Res 2:447–459
Drahota Z, Chowdhury SKR, Floryk D, Mráček T, Wilhelm J, Rauchová H, Lenaz G, Houštěk J (2002) J Bioenegr Biomembranes 34:105–113
Drahota Z, Rauchová H, Ješina P, Vojtíšková A, Houštěk J (2003) Gen Physiol Biophys 22:93–102
Estornell E, Fato R, Pallotti F, Lenaz G (1993) FEBS Lett 332:127–131
Fato R, Estornell E, Di Bernardo S, Pallotti F, Parenti Castelli G, Lenaz G (1996) Biochemistry 35:2705–2716
Genova ML, Merlo Pich M, Biondi A, Bernacchia A, Falasca A, Bovina C, Formiggini G, Parenti Castelli G, Lenaz G (2003) Exp Biol Med 228:506–513
Geromel V, Darin N, Chrétien D, Bénit P, DeLonlay P, Röting A, Munnich A, Rustin P (2002) Mol Genet Metab 77:21–30
Gnaiger E, Steinlechner-Maran R, Mendez G, Eberl T, Margreiter R (1995) J Bioenerg Biomembranes 27:583–596
Houštěk J, Drahota Z (1975) Mol Cell Biochem 7:45–50
James AM, Cocheme HM, Smith RA, Murphy MP (2005) J Biol Chem 280:21295–21312
Kröger A, Klingenberg M (1973) Eur J Biochem 34:358–368
Lenaz G, Bovina C, Castelluccio C, Cavazzoni M, Estornell E, Fato R, Huertas JR, Merlo Pich M, Pallotti F, Parenti Castelli G, Rauchová H (1995) Protoplasma 184:50–62
Lenaz G, Fato R, Formiggini G, Genova ML (2007) Mitochondrion 7(Suppl 1):S8–S33
Linnane AW, Eastwood H (2006) Ann NY Acad Sci 1067:47–55
Lowry OH, Rosenbrough NJ, Farr AL, Randall LJ (1951) J Biol Chem 143:265–275
MacDonald MJ, Husain RD, Hoffmann-Benning S, Baker TR (2004) J Biol Chem 279:27278–27285
Quinzii CM, DiMauro S, Hirano M (2007) Neurochem Res 32: 723–727
Rauchová H, Drahota Z (1984) Int J Biochem 16:243–245
Rauchová H, Battino M, Fato R, Lenaz G, Drahota Z (1992) J Bioenerg Biomembranes 24:235–241
Rauchová H, Fato R, Drahota Z, Lenaz G (1997) Arch Biochem Biophys 344:235–241
Rauchová H, Drahota Z, Lenaz G (2001) In: Ebadi M, Marwah J, Chopra R (eds) Mitochondrial ubiquinone (Coenzyme Q): bio-

chemical, functional, medical, and therapeutic aspects in human health and diseases. Prominent Press, Arizona, pp 217–229
Rauchová H, Drahota Z, Rauch P, Fato R, Lenaz G (2003) Acta Biochim Pol 50:405–413
Rauchová H, Vrbacký M, Bergamini C, Fato R, Lenaz G, Houštěk J, Drahota Z (2006) Biochem Biophys Res Commun 339:362–366
Rustin P, Bonnet D, Rötig A, Munnich A, Sidi D (2004) Neurology 62:524–525
Sohal RS, Forster MJ (2007) Mitochondrion 7(Suppl 1):S103–S111
Turrens JF (2003) J Physiol 552:335–344
Turunen M, Olsson J, Dallner G (2004) Biochem Biophys Acta 1660:171–199
Unemoto T, Hayashi M (1989) J Bioenerg Biomembranes 21:649–662
Vrbacký M, Drahota Z, Mráček T, Vojtíšková A, Ješina P, Stopka P, Houštěk J (2007) Biochim Biophys Acta 1767:989–997
Zs-Nagy I (1990) Arch Gerontol Geriatr 11:177–186