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The Antihypertensive Drug Carvedilol Inhibits the Activity of Mitochondrial NADH-Ubiquinone Oxidoreductase

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A study is presented on the interaction of carvedilol with mitochondria isolated from several rat organs. It is shown that carvedilol causes a moderate uncoupling effect under non phosphorylating succinate supported respiration of intact mitochondria, as well as a marked inhibition of coupled respiration with NAD-dependent substrates. The inhibitory effect was also found in the bovine heart purified Complex I as well as in experiments with mitochondrial particles, where the individual redox segments of the respiratory chain were analysed. It is also shown that carvedilol, though exhibiting an intrinsic scavenger activity, caused reactive oxygen species to be produced as a consequence of its inhibitory effect on the steady-state respiration. Under these conditions the pro-oxidant activity of carvedilol appears to prevail over its scavenging activity, and a net generation of ROS is promoted.

KEY WORDS: Mitochondria; respiratory chain; Complex I; carvedilol; reactive oxygen species; β-blockers; antihypertensive drugs.

INTRODUCTION

Carvedilol (Fig. 1) is an antihypertensive drug, clinically used for the treatment of congestive heart failure and myocardial infarction. It competitively blocks β_1, β_2, and α_1 adrenergic receptors and displays vasodilating properties through the blockade of α_1 receptor (Dunn *et al.*, 1997). Carvedilol has been also repeatedly reported to have antioxidant properties (Santos and Moreno, 2001; Tadolini and Franconi, 1998; Yue *et al.*, 1992a,b, 1994; Yue and Feuerstein, 1992). Thus it is though to protect from the deleterious actions of free radicals, which appear involved in the onset of pathological conditions, particularly concerning the cardiovascular system.

Mitochondrial respiration has long been recognized to be the major source of reactive oxygen species (ROS) (Boveris *et al.*, 1972; Turrens *et al.*, 1985; Turrens and Boveris, 1980). The process of ROS generation by mitochondria is actually enhanced under certain physiopathological conditions, such as tissue ischemia and reperfusion (Gonzales-Flecha *et al.*, 1993; Nohl *et al.*, 1993; Turrens *et al.*, 1991; Vanden Hoek *et al.*, 1997). Among the various events taking place under these conditions, there is a substantial increase of the concentration of long chain free fatty acids, arachidonic acid in particular (see Van der Vusse *et al.*, 1992 for review), which, by inhibiting mitochondrial respiration, promote in turn further ROS generation (Cocco *et al.*, 1999). Furthermore, the high content of polyunsaturated fatty acids in mitochondrial membrane phospholipids makes these membranes susceptible to lipid peroxidation, with consequent loss of mitochondrial functional integrity.

These observations have addressed studies on the interaction of carvedilol with mitochondria, under

Key to abbreviations: ROS, reactive oxygen species; CCCP, carbonyl cyanide m-chlorophenylhydrazone; DCFH-DA, dichlorofluorescin-diacetate; DCFH, dichlorofluorescin; HRP, horseradish peroxidase; PMSF, phenylmethylsulphonylfluoride; DBH, decylubiquinone; TEMED, N,N,N',N'-*p*-phenylediamine; $\Delta\psi$, transmembrane potential.

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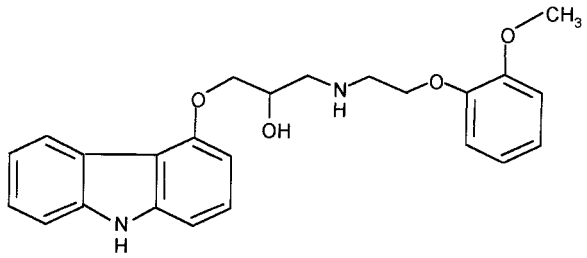

Fig. 1. Structure of carvedilol 1-(carbazol-4-yloxy-3-{[2-(*o*-methoxyphenoxy)ethyl]amino}-2-propanol.

conditions of induced oxidative stress. In several works reporting the antioxidant effect of carvedilol, lipid peroxidation was induced by ADP/Fe^{2+} addition to isolated mitochondria (Abreu *et al.*, 2000; Santos and Moreno, 2001). Under these conditions carvedilol was indeed found to curtail lipid peroxidation and to preserve mitochondria from the consequent drop of membrane potential ($\Delta\Psi$). This effect has been ascribed mainly to the iron-chelating properties of carvedilol (Oettl *et al.*, 2001), therefore, a direct evidence of a radical scavenger activity of carvedilol towards ROS generated in mitochondria is lacking.

Experiments carried out to study the effect of carvedilol on mitochondrial respiration have produced different results. In mitochondria isolated from rat liver, concentrations of carvedilol up to 40 μM, did not cause any variation on either State 4 or State 3 respiration, with succinate as substrate (Abreu *et al.*, 2000). In succinate oxidizing mitochondria from rat heart, low concentrations (up to 10 μM) did not (Santos and Moreno, 2001), whereas higher concentrations (till 100 μM) did cause increase of State 4 respiration (Oliveira *et al.*, 2000). Subsequently the latter effect was associated to a protonophoric mechanism exerted by carvedilol, leading to a partial dissipation of membrane potential ($\Delta\Psi$) (Oliveira *et al.*, 2000, 2001).

In this work we present experiments aimed at analysing the interaction of carvedilol with the components of the respiratory chain in mitochondria isolated from several rat organs. The data we present show that (i) carvedilol at the concentrations commonly used for *in vitro* experiments inhibits markedly the activity of Complex I under phosphorylating or uncoupled conditions; (ii) the inhibition of Complex I activity is accompanied by a substantial increase of the rate of ROS production; (iii) carvedilol exhibits an intrinsic ROS scavenger activity, but, as a consequence of the inhibition exerted on the steady-state respiration, its pro-oxidant effect appears to prevail over its scavenging activity.

MATERIALS AND METHODS

Chemicals

2′7′-Dichlorofluorescin-diacetate (DCFH-DA) was obtained from Eastmann Kodak (Rochester, NY). Antimycin A, rotenone, phenylmethylsulphonylfluoride (PMSF), safranin-O, decylubiquinone (DBH), horse-heart cytochrome *c* (type VI) from Sigma Chemical Co. (St. Louis, MO). Horseradish peroxidase (HRP) was obtained from Boehringer, Mannheim. Carvedilol was a gift of Dr. Paulo J. Oliveira, Department of Zoology, Universidade de Coimbra, Portugal. Carvedilol was dissolved in dimethyl sulphoxide (DMSO) as 10 mM solution and stored frozen. All other reagents were of the highest purity grade commercially available.

Preparation of Heart and Liver Mitochondria

Rat heart and liver mitochondria were isolated by differential centrifugation of tissue homogenate. Briefly, hearts from male Wistar rats (200–250 g) were placed in ice-cold 0.25 M sucrose, pH 7.4, quickly excised and ventricles, carefully devoided of fat and connective tissue, were then finely minced and homogenized in 10 volumes of isolation medium containing 0.25 M sucrose, 10 mM Tris–Cl, pH 7.4, 1 mM EGTA, 0.25 mM PMSF. Liver tissue was minced and homogenized in 10 volumes of isolation medium containing 0.25 M sucrose, 10 mM Hepes–KOH, pH 7.4, 1 mM EGTA, 0.25 mM PMSF. The homogenate of both tissues was centrifuged at 1200*g* for 10 min. The resulting supernatant was centrifuged at 9500*g* for 10 min and the pellet, resuspended in the same buffer, was centrifuged at 14000*g* for 10 min. The pellet was washed gently to remove any light or loosely packed damaged mitochondria, resuspended in the isolation buffer and centrifuged again as above. The final pellet was resuspended in the respective isolation medium at a protein concentration of 50–60 mg/mL as determined by the Biuret method. All the centrifugation steps were carried out at 0–4°C.

Preparation of Brain Mitochondria

For free non-synaptic mitochondria preparation, the isolation method described by Nagy and Delgado-Escueta (1984) was used, with some minor modifications. The brain regions were washed with STE medium (0.32 M sucrose, 10 mM Tris, 0.1 mM EDTA, pH 7.4) and homogenized with 10 volumes of the same medium. The nuclei were separated by centrifugation at 1000*g* for 10 min. The supernatant was centrifuged at 12000*g* for 20 min to

obtain the crude mitochondrial pellet. The pellet was washed twice and suspended in STE buffer. To each milliliter of crude mitochondrial suspension, 7.5 mL of a 8.5% Percoll solution in 0.25 M sucrose were added. The final suspension was layered on the top of a 10–20% discontinuous Percoll/sucrose gradient. After centrifugation at 25000*g* for 20 min, the free mitochondria were obtained as a pellet at the bottom of the tube. The pellet was washed three times with STE buffer by centrifugation at 20000*g* for 7 min, to remove the residual Percoll. The final pellet was suspended in STE buffer.

Isolation of NADH-Ubiquinone Oxidoreductase

NADH-ubiquinone oxidoreductase (Complex I) was isolated from bovine heart mitochondria as described by Hatefi (1978) and stored at -80°C in TSH buffer (50 mM Tris–Cl, pH 8.0, 0.67 M sucrose, 1 mM Histidine).

Measurement of Oxygen Consumption Rate

The respiratory activity of freshly prepared mitochondria was measured polarographically in a Rank Brothers oxygraph by suspending mitochondria (0.25 mg/mL) at 25°C. Rat heart mitochondria were suspended in a medium containing 75 mM sucrose, 50 mM KCl, 30 mM Tris–Cl, pH 7.4, 5 mM KH_2PO_4, 0.5 mM EDTA, 2 mM $MgCl_2$. Rat liver mitochondria were suspended in 0.25 M sucrose, 50 mM Hepes–KOH, pH 7.4, 10 mM KH_2PO_4, 4 mM $MgCl_2$. Rat brain mitochondria were suspended in 300 mM mannitol, 10 mM Tris–Cl, pH 7.4, 0.2 mM EDTA, 10 mM KH_2PO_4. State 4 respiration was started by the addition of pyruvate (3.5 mM)/malate (1.7 mM) or succinate (7 mM) in the presence of 1μg/mL rotenone, or ascorbate (1.4 mM)/TMPD (0.4 mM) in the presence of 1.2 μM antimycin A. State 3 and uncoupled respiration were obtained by adding 1 mM ADP and 0.2 μM CCCP, respectively.

Measurement of Redox Activities of the Individual Respiratory Complexes in Rat Heart, Liver, and Brain Mitochondrial Particles

Enzyme activities were measured by suspending 0.25 mg/mL of mitochondrial particles, prepared by freezing and thawing (three times) of mitochondria isolated from rat heart, liver, and brain, in a medium containing 50 mM K-phosphate buffer, pH 7.4, 25 μM EDTA (final volume 1.6 mL), at 25°C.

NADH-CoQ oxidoreductase (Complex I) and succinate-cytochrome *c* oxidoreductase (Complex II+III) activities were determined spectrophotometrically with a double-beam, dual-wavelength spectrophotometer (Johnson Research Foundation, Philadelphia) as reported previously (Cocco *et al.*, 1999).

Cytochrome *c* oxidase (Complex IV) activity was measured as cyanide-sensitive ascorbate/TMPD oxidase activity. Oxygen consumption was started by the addition of 10 mM ascorbate/0.4 mM TMPD (in the presence of 1.2 μM antimycin A) to mitochondrial particles suspension supplemented with 0.2 μM cytochrome *c*.

Measurement of the Redox Activity of Complex I Isolated From Bovine Heart Mitochondria

The redox activity of purified Complex I was measured spectrophotometrically by following the rotenone-sensitive initial rate of NADH oxidation at 360–374 nm ($\Delta\varepsilon = 2.3\ \mathrm{mM}^{-1} \cdot \mathrm{cm}^{-1}$). Purified enzyme (30 μg) was suspended in a reaction mixture (final volume 1.6 mL) consisting of 10 mM K-phosphate buffer, pH 8.0, 0.15 mg/mL sonicated soybean phospholipids, 100 μM decylubiquinone, and 1 mM azide, at 30°C. The reaction was started by the addition of 60 μM NADH.

Measurement of Membrane Potential

The membrane potential in intact mitochondria was measured following the safranin fluorescence quenching at 525 nm (excitation), 575 nm (emission) with a Perkin Elmer 650 fluorescence detector.

Freshly prepared mitochondria (0.25 mg protein/mL) were suspended in the same medium described for oxygen consumption experiments, supplemented with 8 μM safranin, at 25°C. The transmembrane potential was generated by the addition of respiratory substrates or 1.5 mM ATP (Di Paola *et al.*, 2000).

Measurement of Mitochondrial H_2O_2 Production

The rate of mitochondrial hydrogen peroxide production was estimated by measuring the linear fluorescence increase (excitation at 475 nm, emission at 525 nm) caused by the H_2O_2-dependent oxidation of dichlorofluorescin (DCFH) to the fluorescent compound dichlorofluorescein, in the presence of horseradish peroxidase (HRP) (Black and Brandt, 1974; Cocco *et al.*, 1999). Immediately prior to determinations, DCFH was obtained from the stable reagent dichlorofluorescin-diacetate (DCFH-DA) by alkaline treatment (Black and Brandt, 1974).

Rat heart and liver mitochondria were suspended in the same medium described for oxygen consumption

determinations, supplemented with 0.4 μM HRP and 5 μM DCFH. Pyruvate+malate or succinate were used as substrates. Conversion of fluorescence units to nmol of H_2O_2 produced was performed by measuring the fluorescence changes upon addition of known amounts of H_2O_2.

RESULTS

Effect of Carvedilol on the Respiratory Chain Redox Reactions

The effect of carvedilol on oxygen consumption by mitochondria isolated from rat heart was examined using pyruvate+malate or succinate as substrates. With pyruvate+malate (Fig. 2(a)), carvedilol, while not affecting apparently the rate of oxygen consumption under

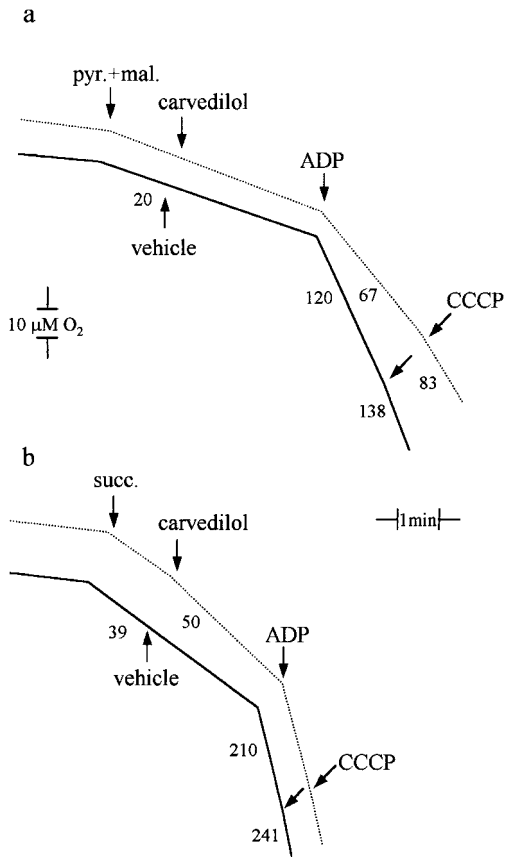

Fig. 2. Effect of carvedilol on mitochondrial oxygen consumption. Rat heart mitochondria were suspended in the reaction medium described under Materials and Methods. Oxygen consumption was initiated by the addition of pyruvate+malate (a) and succinate (in the presence of rotenone) (b). A 20 μM carvedilol (dotted traces, vehicle in the control) was added, followed by the addition of 1 mM ADP and 0.2 μM CCCP. Numbers on the traces refer to the rate of oxygen consumption as nm of $O_2 \cdot min^{-1} \cdot mg\ protein^{-1}$.

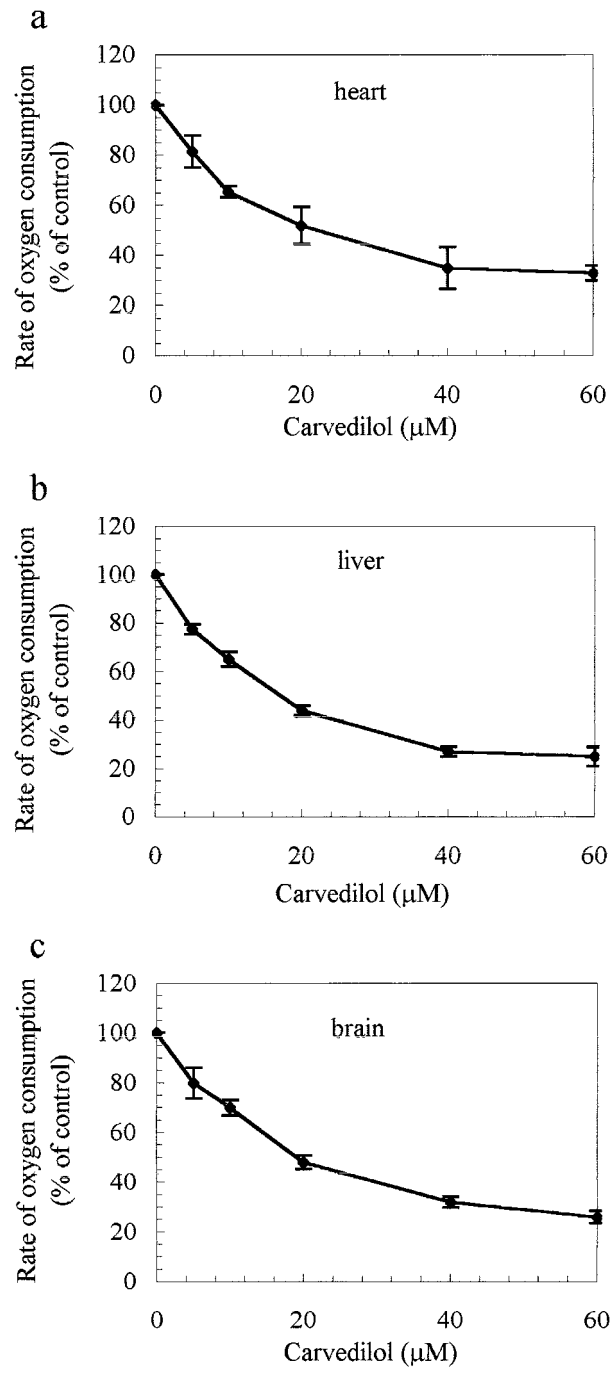

Fig. 3. Effect of carvedilol on mitochondrial State 3 respiration. Freshly prepared heart (a), liver (b), and brain (c) mitochondria were suspended in the reaction media described under Materials and Methods. Respiration was started by the addition of pyruvate+malate followed by 1 mM ADP. Control values for State 3 respiration were 115.4 ± 5.1, 64.6 ± 4.2, 61.3 ± 4.1 nmol of $O_2 \cdot min^{-1} \cdot mg\ protein^{-1}$ for heart, liver, and brain mitochondria, respectively. The values reported represent the means $\pm$ SD of 10 measurements from five different experiments.

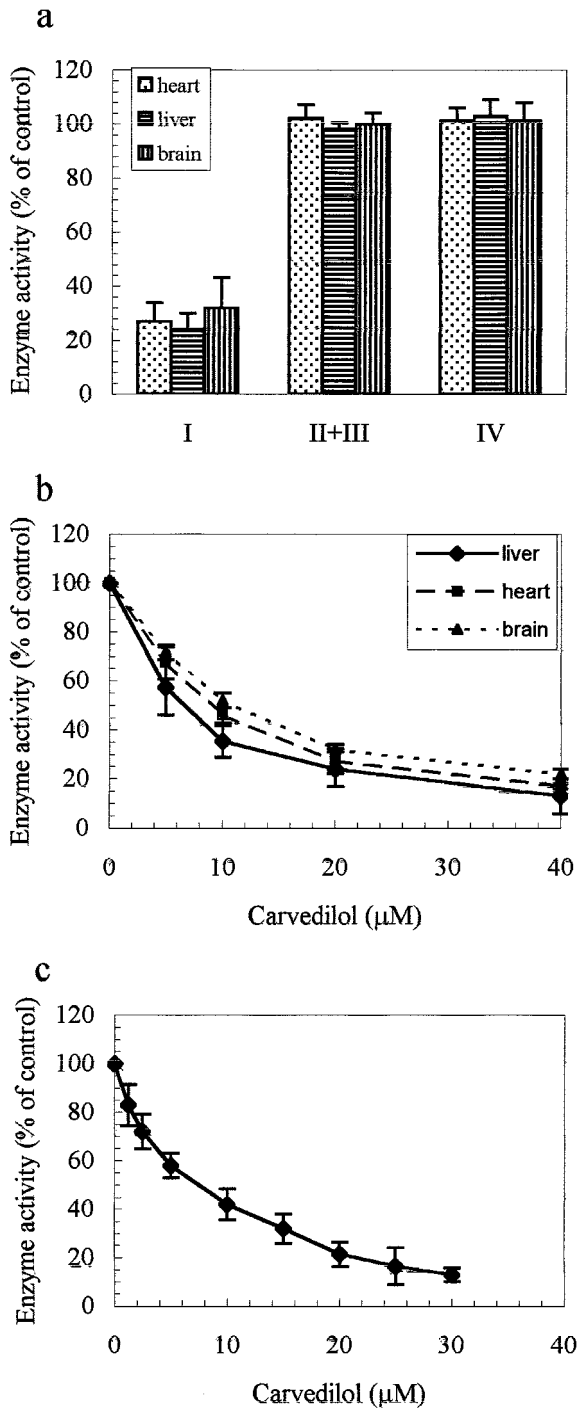

Fig. 4. Effect of carvedilol on the activity of mitochondrial respiratory complexes. The determinations of enzyme activities in mitochondrial particles from rat heart, liver, and brain were performed as reported under Materials and Methods. (a) Effect of 20 μM carvedilol on Complex I, II+III, and IV activities. The values are the means ± SD of six measurements from three different mitochondrial preparations. Control values for specific activities, as nmol · min^{-1} · mg protein^{-1}, in heart, liver, and brain respectively, were 325.2 ± 7.1, 93.2 ± 6.0, and 142.2 ± 11.2 for Complex I; 299.4 ± 5.2, 111.2 ± 2.4, and 112.2 ± 4.1 for Complex II+III, and 281.9 ± 5.2, 125.4 ± 4.3, and 170.9 ± 7.1 for Complex IV. (b) Concentration dependence of the effect of carvedilol on redox activity of Complex I in rat heart, liver, and brain mitochondrial particles. (c) Effect of carvedilol on redox activity of Complex I isolated from bovine heart mitochondria. Specific activity in the control was 1.92 ± 0.32 μmol · min^{-1} · mg protein^{-1}.

controlled state, caused a marked inhibition of oxygen consumption measured under coupled state (in the presence of ADP). The inhibition, whose extent was around 40% in the presence of 20 μM carvedilol, was not reversed by the uncoupler CCCP.

With succinate as substrate (Fig. 2(b)), carvedilol caused a small stimulation of the rate of oxygen consumption under State 4 respiration (the mean value was 30% ± 7% increase at 20 μM carvedilol, $n = 6$), which is likely associated with its reported protonophoric properties (Oliveira *et al.*, 2000, 2001) (see also below). The rate of oxygen consumption under State 3, or in the presence of the uncoupler, was, on the contrary, unaffected. The inhibition of State 3 or uncoupled respiration, observed when electrons enter the respiratory chain at Site I, indicates that carvedilol inhibits the activity of Complex I and, hence, the respiration supported by NAD-dependent substrates. The apparent lack of inhibition of pyruvate+malate oxidation under controlled state may be due to a compensating uncoupling effect of carvedilol. Separate controls have indeed shown that also under our experimental conditions, carvedilol reduced the transmembrane potential ($\Delta\Psi$) set up by either pyruvate+malate or succinate oxidation as well as by hydrolysis of ATP in mitochondria (not shown).

The concentration dependence of the carvedilol effect on NAD-linked substrate oxidation in phosphorylating mitochondria isolated from three different rat organs is shown in Fig. 3. Respiratory activity in mitochondria from heart (Fig. 3(a)), liver (Fig. 3(b)), and brain (Fig. 3(c)) was largely inhibited by carvedilol. The I_{50} value, the concentration of carvedilol causing 50% inhibition, was around 20 μM for all the mitochondrial preparations being tested.

Since several mechanisms, including substrate transport, may be involved in the inhibition by carvedilol of the respiratory activity, attempts were made to measure the individual redox system activities in mitochondrial particles, obtained by freezing and thawing of isolated mitochondria. The rotenone-sensitive NADH-Q reductase (Complex I) activity from heart, liver, and brain mitochondria was severely inhibited by 20 μM carvedilol (Fig. 4(a)). The succinate-cytochrome *c* reductase (Complex II+III), as well as the ascorbate+TMPD oxidase (Complex IV) activities were unaffected by carvedilol. The concentration

dependence of the effect of carvedilol on NADH-Q reductase activity (Fig. 4(b)) shows that the I_{50} value for the inhibition (8–10 μM) is lower than that found in intact mitochondria. An easier accessibility of carvedilol to enzyme complexes in mitochondrial particles than in intact mitochondria can be argued from this observation. Figure 4(c) shows that the activity of Complex I isolated from bovine heart was also almost completely inhibited by carvedilol, with an I_{50}value of the around 8 μM. Separate experiments have shown that carvedilol did not inhibit the NADH-ferricyanide reductase activity of isolated Complex I. This observation suggests that, like rotenone, carvedilol inhibits Complex I by interaction at the quinone reduction site(s) (see Vinogradov, 1998, for review). The activities of either Complex III or Complex IV, both isolated from bovine heart too, were insensitive to carvedilol (not shown).

Effect of Carvedilol on ROS Generation in Mitochondria

Addition of succinate to mitochondria respiring under non phosphorylating state caused a large $\Delta\psi$-dependent H_2O_2 production (Fig. 5), which was in fact uncoupler-sensitive (Trace 4) and greatly reduced by the addition of ADP (Trace 3) (Boveris and Chance, 1973). Rotenone strongly inhibited this H_2O_2 production (Trace 2) (Hansford *et al.*, 1997; Korshunov *et al.*, 1998; Kwong and Sohal, 1998), and this led to the conclusion that it is mainly coupled to $\Delta\Psi$-driven reverse electron transfer from succinate to Complex I (Korshunov *et al.*, 1998).

The addition of carvedilol caused a concentration-dependent decrease of H_2O_2 production (Trace 1). This effect can be explained by several mechanisms including (i) rotenone-like inhibition by carvedilol of the Complex I activity; (ii) dissipation of the transmembrane potential ($\Delta\Psi$), owing to its protonophoric properties; (iii) a direct carvedilol scavenger activity. In Fig. 6 the results of an experiment are reported, in which the capability of carvedilol to scavenge ROS generated in mitochondria was valued. ROS were generated by the addition of arachidonic acid (AA) (Trace a) (Cocco *et al.*, 1999) and rotenone (Trace b) (Kwong and Sohal, 1998) to aerobic heart mitochondria respiring with pyruvate+malate as substrate. Under these conditions, both compounds inhibited completely Complex I activity (Cocco *et al.*, 1999). Addition of carvedilol caused a substantial decrease of the rate of ROS generation. However, when carvedilol was let to inhibit the steady-state pyruvate+malate supported oxygen consumption, it caused a remarkable increase of ROS production, particularly under State 3 conditions (Fig. 7(a)). Panels b and c in Fig. 7 show a statistical evaluation of the pro-oxidant activity of carvedilol, measured in respiring mitochondria isolated from rat heart and liver,

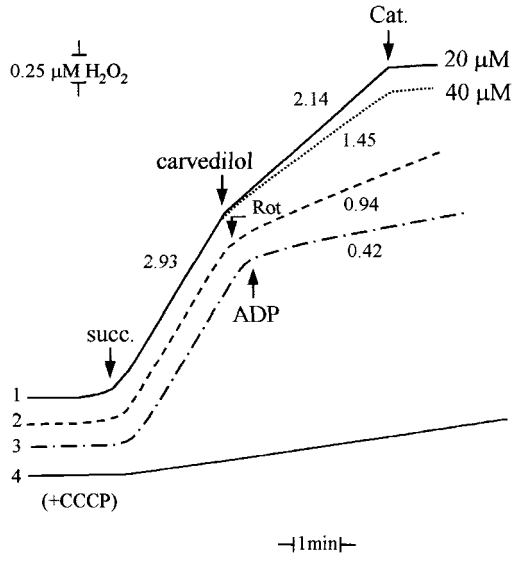

Fig. 5. Effect of carvedilol on H_2O_2 production by $\Delta\psi$-driven reverse electron transfer in rat heart mitochondria. Measurement of H_2O_2 production by mitochondria at the steady-state respiration was performed as described under Materials and Methods. Succinate (14 mM) was used as substrate. Where indicated, 1 mM ADP (Trace 3), 0.8 μg rotenone (Trace 2), 20–40 μM carvedilol (Trace 1) were added. Trace 4 refers to an experiment in which 0.4 μM CCCP was present in the reaction mixture. Figures on the traces refer to the rate of H_2O_2 production as nmol · min^{-1} · mg protein^{-1} · Cat (0.5 μM catalase).

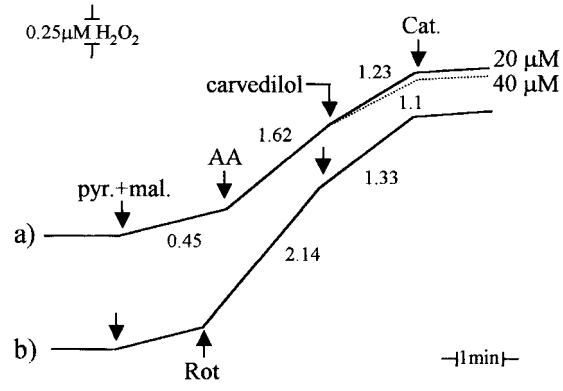

Fig. 6. Scavenger activity of carvedilol towards ROS generation in respiring rat heart mitochondria. Freshly prepared mitochondria were suspended in the reaction medium described under Materials and Methods. Pyruvate+malate was used as substrate. Where indicated, 100 μM arachidonic acid (AA), 0.8 μg rotenone, 20–40 μM carvedilol were added. Figures on the traces refer to the rate of H_2O_2 production as nmol · min^{-1} · mg protein^{-1} · Cat (0.5 μM catalase).

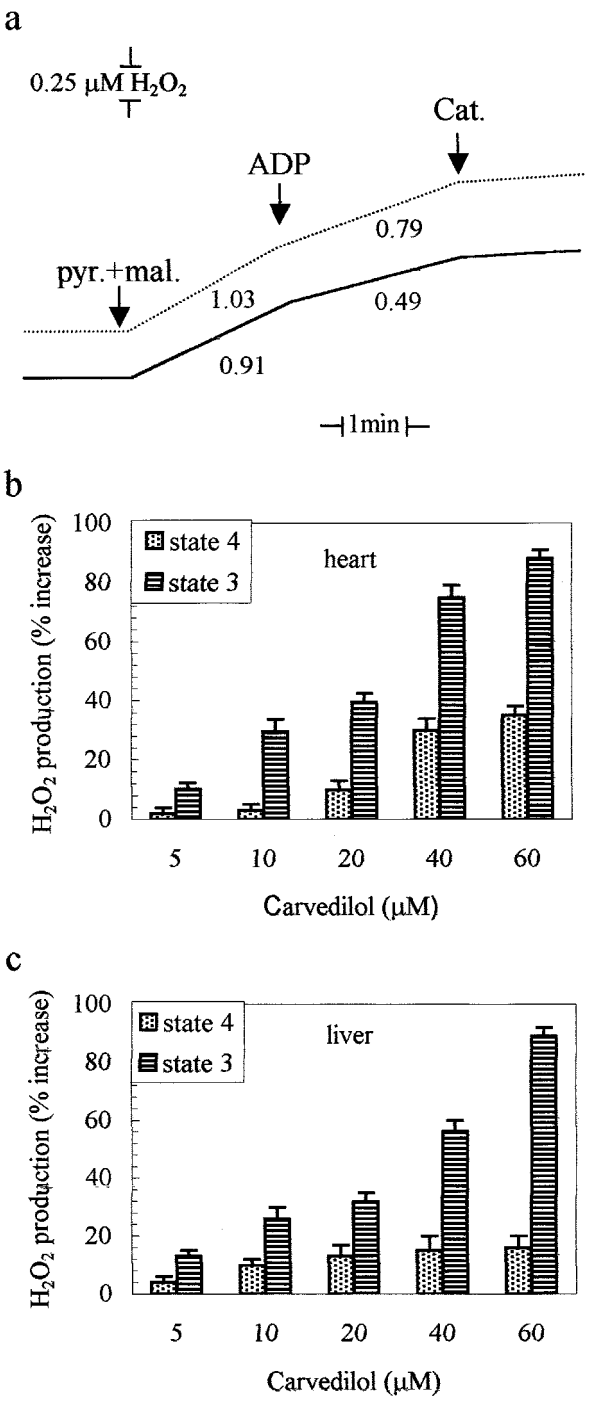

Fig. 7. H_2O_2 generation in mitochondria respiring with pyruvate+malate as substrate. Effect of carvedilol: (a) heart mitochondria were suspended in the reaction mixture, in the presence (dotted line) or in the absence (solid line) of 20 μM carvedilol. Pyruvate+malate and ADP were added at the same concentrations used for the determination of oxygen consumption. Figures on the traces refer to the rate of H_2O_2 production as nmol $\cdot$ min^{-1} $\cdot$ mg protein^{-1}. In (b) and in (c) a statistical evaluation of carvedilol dependent hydrogen peroxide generation increase in respiring heart and liver mitochondria is reported.

respectively. No effect of carvedilol on the rate of ROS generation was detected when succinate (*plus* rotenone) was used as substrate (not shown).

DISCUSSION

When the effect of a drug, such as carvedilol, is studied at subcellular level, the question arises whether it can accumulate into the cell to reach the concentrations used *in vitro* experiments. It is relevant considering that a plasma concentration of 0.3 μM carvedilol has been measured after an oral dose of 50 mg (Yue *et al.*, 1992b). However, as argued by Oliveira *et al.* (2001), carvedilol, owing to its great lipophilicity, may reach membrane concentrations several times higher, thus falling within the range used here and by others (Abreu *et al.*, 2000; Oliveira *et al.*, 2000, 2001; Santos and Moreno, 2001).

The results we have obtained show that carvedilol induces toxic effects on mitochondrial functions, since it inhibits the activity of respiratory chain Complex I and the respiration supported by NAD-dependent substrates. This inhibition, also found with the Complex I isolated from bovine heart, was observed in mitochondrial preparations from several rat tissues. Thus, the inhibitory effect of carvedilol can be considered systemic.

In order for the reported antioxidant properties of carvedilol to be evaluated, experiments were performed, in this study, in which ROS were generated through the $\Delta\Psi$-dependent reverse electron transfer from succinate to Complex I (Fig. 5). Carvedilol did readily reduce the rate of ROS generation under these conditions. Whether this effect is due to carvedilol uncoupling properties or to its capability to scavenge ROS directly, cannot be inferred from this experiment. However, the results presented in Fig. 6 allow to ascertain, for the first time, that carvedilol displays an intrinsic scavenger activity. In fact, when ROS are generated under conditions in which electron flow through the respiratory chain is inhibited and the $\Delta\Psi$ is absent (Fig. 6), carvedilol did cause a significant decrease of the rate of ROS generation. It is worth considering that the conditions of this experiment resemble those of the ischemia/reperfusion process, characterized by an high reduction level of intermediate electron carriers of the mitochondrial respiratory chain. However, under respiratory steady-state conditions, inhibition by carvedilol of Complex I activity, appears to be associated to a substantial increase of the rate of ROS generation (Fig. 7). The net process of ROS production observed under these conditions is likely resulting from the difference between the carvedilol prooxidant activity and its intrinsic scavenger activity. Such a paradoxical behaviour of an antioxidant,

which promotes ROS generation, has also been shown to be exhibited by resveratrol, which interacts with the mitochondrial respiratory chain as well (Tinhofer *et al.*, 2001; Zini *et al.*, 1999).

These toxic effects of carvedilol, which appear to involve several organs like heart, liver, or brain, have to be considered when the drug is administered for clinical use. On this ground a recently reported hepatotoxicity associated with carvedilol treatment (Hagmeyer and Stein, 2001) can be explained.

ACKNOWLEDGMENTS

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