J Bioenerg Biomembr (2009) 41:387–393
DOI 10.1007/s10863-009-9238-2

Succinate is the controller of O_2^-/H_2O_2 release at mitochondrial complex I : negative modulation by malate, positive by cyanide

Franco Zoccarato · Lucia Cavallini · Adolfo Alexandre

Received: 6 July 2009 / Accepted: 25 August 2009 / Published online: 15 September 2009
© Springer Science + Business Media, LLC 2009

Abstract Mitochondrial production of H_2O_2 is low with NAD substrates (glutamate/pyruvate, 3 and 2 mM) (G/P) and increases over ten times upon further addition of succinate, with the formation of a sigmoidal curve (semimaximal value at 290 μM, maximal H_2O_2 production at 600 μM succinate). Malate counteracts rapidly the succinate induced increased H_2O_2 release and moves the succinate dependent H_2O_2 production curve to the right. Nitric oxide (NO) and carbon monoxide (CO) are cytochrome c oxidase inhibitors which increase mitochondrial ROS production. Cyanide (CN^-) was used to mimic NO and CO. In the presence of G/P and succinate (300 μM), CN^- progressively increased the H_2O_2 release rate, starting at 1.5 μM. The succinate dependent H_2O_2 production curve was moved to the left by 30 μM CN^-. The V_{max} was little modified. We conclude that succinate is the controller of mitochondrial H_2O_2 production, modulated by malate and CN^-. We propose that succinate promotes an interaction between Complex II and Complex I, which activates O_2^- production.

Keywords Reactive oxygen species · Brain mitochondria · Complex I · Succinate · Malate · Nitric oxide · Carbon monoxide · Calorie restriction · Hyperglycemia

Abbreviations

ROS	reactive oxygen species
O_2^-	superoxide
NO	nitric oxide
CO	carbon monoxide
CCO	cytochrome c oxidase
CN^-	cyanide
OAA	oxaloacetate
SOD	superoxide dismutase
CoQ	coenzyme Q
Cpl I (II)	Complex I (II)
CR	calorie restriction
HRP	horseradish peroxidise
BSA	bovine serum albumin
Mops	4-morpholinepropanesulfonic acid

F. Zoccarato · L. Cavallini · A. Alexandre (✉)
Dipartimento di Chimica Biologica, Università di Padova,
Viale Giuseppe Colombo 3,
35131 Padova, Italy
e-mail: adolfo.alexandre@unipd.it

Introduction

Complex I (Cpl I) of the mitochondrial respiratory chain is a 46 subunit protein of over 1000 kDa mass (Brandt 2006) which, besides being involved in proton pumping associated with electron transfer from NADH to CoQ, is the main site of ROS generation in mitochondria (reviewed in Murphy 2009; Starkov 2008). The species being generated by one-electron reduction of oxygen is superoxide (O_2^-), which is released towards the intramitochondrial space. O_2^- is transformed into H_2O_2 by Mn-superoxide dismutase (SOD) also located intramitochondrially and the highly permeable H_2O_2 exits from the mitochondria. It is measured in the extramitochondrial space monitoring the horseradish peroxidise driven oxidation of Amplex Red to the fluorescent resorufin. The rate of H_2O_2 generation is highly variable. It is low with NAD linked substrates, which lead to CoQ reduction via Cpl I ; alternatively, succinate determines a high rate of H_2O_2 production (Murphy 2009; Starkov 2008; Zoccarato et al. 2007; Muller et al. 2008). Succinate is oxidized by succinate dehydrogenase which feeds electrons to CoQ following a different

path, via Complex II (Cpl II). However the site of O_2^-/H_2O_2 generation with succinate is attributed to Cpl I, being inhibited by the Cpl I inhibitor rotenone. Succinate-dependent H_2O_2 generation is sensitive to the decrease of mitochondrial membrane potential. These properties, together with the general habit in the literature of using high concentrations of succinate as the sole substrate led to the idea that the high rate of H_2O_2 generation with succinate depends on a backwards electron flux into Cpl I of little physiological relevance. The electron path supposedly being followed was

Succ → Cpl II → CoQ → Cpl I → NADH

with O_2^- being produced in the CoQ→Cpl I→NADH step, energized by the protonmotive force generated by succinate oxidation. Some recent observations from this laboratory suggest that the things may not work like this: first of all, small succinate concentrations suffice to generate H_2O_2, and such H_2O_2 generation is essentially unmodified by the contemporary oxidation of NAD-linked substrates (glutamate/malate) (Zoccarato et al. 2007; Muller et al. 2008; Zoccarato et al. 2005). Secondly, the physiological electron flux in Cpl I in the direction NADH→CoQ is slowed down but not totally prevented by succinate (i.e. there is no net reverse electron transfer) as shown by uninterrupted production of α-ketoglutarate, from glutamate/malate, in the presence of succinate (Zoccarato et al. 2007). It is to conclude that a downward electron flow in Cpl I and Cpl II is always present, also during H_2O_2 generation. The H_2O_2 production with low succinate in the presence of high NAD-linked substrates was recently confirmed in (Muller et al. 2008) that also described an inhibitory effect of malate on the succinate dependent H_2O_2 production.

We reported recently that the succinate generated H_2O_2 is inhibited by palmitoyl-CoA (Bortolami et al. 2008) and by some propargylamine containing compounds (Zoccarato et al. 2008). In this study the properties of succinate induced H_2O_2 production (succ→H_2O_2) are described together with the action of a negative (malate) and a positive (cyanide, CN^-) controller. The experiments were from brain mitochondria. Similar results were obtained with heart mitochondria.

Materials and methods

Reagents Amplex Red (10-acetyl-3,7-dihydroxyphenoxazine) was from Molecular Probes. HRP (horseradish peroxidise; grade I ; EC 1.11.1.7) and all chemicals were from Sigma.

Animals Rats (150–200 g) were housed in temperature-controlled cages (20–22°C), fed ad libitum, and maintained on a 12:12 light/dark cycle. Study design and experimental protocols of research project was in accord with animal welfare and protection and approved by Veterinary Central Services of Padua University.

Preparation of rat brain mitochondria Brain mitochondria were isolated from the cerebral cortices by discontinuous Ficoll density gradient centrifugation as described in (Zoccarato et al. 2008).

Standard incubation method Mitochondria (0.5–0.8 mg/ml) were incubated at 30°C in 125 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgCl_2$, 500 µg/ml defatted BSA, 20 mM Mops, pH 7.3 (adjusted with KOH) and 100 µM EGTA. Further additions were as specified in the figure legends.

H_2O_2 measurements H_2O_2 was measured with 7 µM Amplex Red and 12.5 µg/ml HRP (3 units) included in the incubations. H_2O_2 was detected by the formation of the fluorescent Amplex Red oxidation product resorufin using excitation and emission wavelengths of 563 and 587 nm respectively on a Shimadzu RL-5000 spectrofluorometer in a stirred cuvette as in (Zoccarato et al. 2005). The H_2O_2 calibration scale is linear in the 0–3.5 µM range, and at the end of each assay, traces were calibrated by the addition of H_2O_2 (500 p moles). CN^- is an inhibitor of the heme enzyme HRP. At the low CN^- concentrations used in this study no significant inhibition was observed: however, in the experiments with CN^- (and in their controls) HRP was increased to 31 µg/ml (7.5 units).

Other assays Mitochondrial respiration was monitored in the presence of HRP and Amplex Red with a Clark-type oxygen electrode in a 1.6 ml closed chamber with continuous stirring.

Results

Succinate promotes a sigmoidal increase of H_2O_2 production We first of all performed a succinate titration of the H_2O_2 production in brain mitochondria by supplementing increasing succinate concentrations to mitochondria already receiving a high NAD substrate supply, in the form of glutamate plus pyruvate (G/P), 3 and 2 mM respectively. The H_2O_2 release with G/P was constant at 170 pmoles/mg/min (the release was slightly lower with glutamate alone). In this situation, succinate increased H_2O_2 release beginning at very low concentrations (about 15 µM); the output increased slowly till a concentration of 100–150 µM was reached (Fig. 1 inset). From this point the H_2O_2 release increased more sharply reaching the maximum at about 600 µM. The half maximal generation was at

approximately 290 µM succinate, with the formation of a sigmoidal curve (Fig. 1). The H_2O_2 release rates were constant for some minutes. In some mitochondrial preparations the H_2O_2 output rates slowed down at 2–3 min probably due to the accumulation of inhibitory malate (see below). The H_2O_2 release enhancing effect of succinate was largely but not completely abolished by the addition of ADP, which depolarizes the inner mitochondrial membrane (Fig. 1). This experiment shows that succinate, a tricarboxylic acid (TCA) cycle substrate, is a general controller of mitochondrial H_2O_2 release. The result is particularly significant in view of the recent finding that in hyperglycemia the renal succinate concentration increases sharply from a low value of 10 µM to over 600 µM (Toma et al. 2008) (thus promoting renin release via the specific receptor GPR91). This variation of succinate concentration spans the entire range of concentrations in Fig. 1 and indicates that mitochondria are probably the site of origin of the ROS species being generated in hyperglycemia, via increased succinate. Mitochondria have been previously suggested to be the site of origin of H_2O_2 in diabetes (Nishikawa et al. 2000; Brownlee 2005).

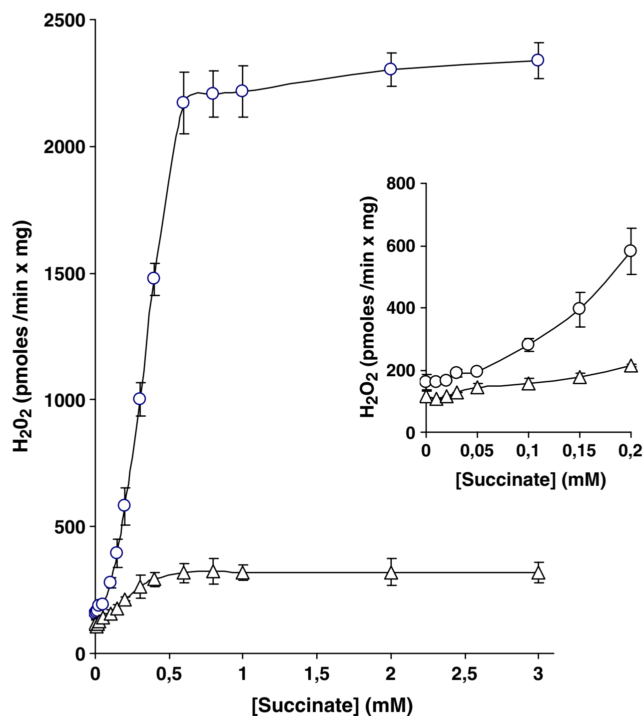

Fig. 1 Succinate promotes the increase of H_2O_2 production. Mitochondria (0.6 mg/ml) were incubated with glutamate (3 mM) and pyruvate (2 mM) (G/P) plus increasing concentrations of succinate. H_2O_2 generation was measured with HRP and Amplex red as reported in experimental procedures. After 3–5 min incubation ADP (1.2 mM) was added. Final volume 1.6 ml. The rates of H_2O_2 production without (o) and with (Δ) ADP are reported. **Inset**: H_2O_2 production at the lowest succinate concentrations. Value are the means ± S.D. from at least seven independent experiments

Malate counteracts the effect of succinate It was published recently that malate decreases the H_2O_2 production induced by succinate in the presence of glutamate (Muller et al. 2008; Starkov 2008). The inhibition was ascribed to the generation of oxaloacetate (OAA), a well known inhibitor of succinate dehydrogenase (Huang et al. 2006; Kearney et al. 1972). Also in our hands malate caused an inhibition of succinate induced H_2O_2 generation. When the H_2O_2 production was measured in the conditions of Fig. 1 (G/P plus a fixed 300 µM succinate) the extra addition of increasing malate induced a decrease of H_2O_2 production as shown in Fig. 2a. If a fixed (1 mM) malate concentration was chosen, and a succinate titration of H_2O_2 production was performed in the presence of G/P, a sigmoidal curve was obtained, similar to that of Fig. 1, but moved to higher succinate concentrations (Fig. 2b). The Vmax of H_2O_2 release appeared to be also decreased by malate. It is not clear from these experiments whether malate is the real inhibitory species. Anyway, if during the progress of H_2O_2 production from G/P and succinate (300 µM), malate was added, it determined a sudden decrease of the H_2O_2 production rate, with the immediate attainment of the final velocity also with the lowest malate concentration (Fig. 2c). The addition of fumarate in place of malate induced a slow decrease of H_2O_2 production (not shown). Adding aspartate had no effect (not shown). OAA appears therefore to be the only possible inhibitor in alternative to malate. The addition of malate (1 mM) to mitochondria oxidizing G/P plus 0.5 mM succinate decreased slightly (from 16.1±0.15 to 13.8±0.10 natoms oxygen/min x mg, n=3) the respiration rate, which may indicate the formation of some OAA. In the same conditions (Fig. 2b) malate inhibited H_2O_2 production by about 60%. So at this point we can say that malate acts quickly, but it is not clear whether the effect is malate or OAA dependent.

Low cyanide concentrations potentiate the effect of succinate It is generally agreed that mitochondrial ROS production decreases with decreasing [O_2] (Murphy 2009; Turrens 2003). However it was recently understood that in conditions of very low O_2 tension there is a paradox increase of mitochondrial H_2O_2 generation (Murphy 2009; Turrens 2003). Nitric oxide (NO) and carbon monoxide (CO) are physiological mediators whose function, besides activation of soluble guanylate cyclase, is to inhibit cytochrome c oxidase (CCO) (Beltrán et al. 2002; Palacios-Callender et al. 2004; Zuckerbraun et al. 2007). Accurate studies on the action of NO on cellular respiration indicate that its physiological effect is to decrease the O_2 sensitivity of CCO (Palacios-Callender et al. 2007). It was shown that in the presence of physiological NO concentrations a state of reduction of cytochrome c and CCO was induced before a sizeable inhibition of respiration could be observed: the

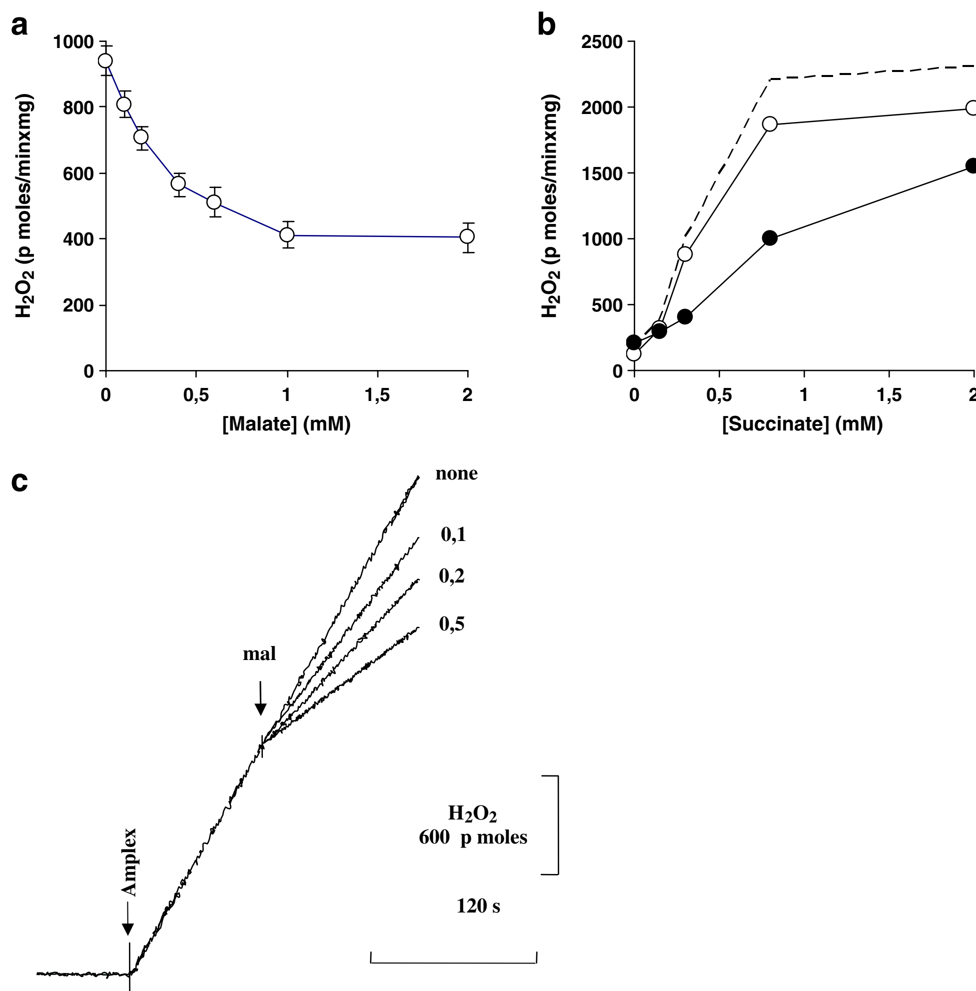

Fig. 2 Negative modulation by malate of the succinate induced H_2O_2 production. **a** H_2O_2 production from mitochondria incubated with G/P and 300 μM succinate plus variable malate concentrations. Value are the means ± S.D. from at least four experiments. **b**Titration of the succ→H_2O_2 production in the presence of G/P, without (o) and with (●) malate (1 mM). The points were taken from the same mitochondrial preparation. The experiment was repeated twice. The dotted line reports the values in Fig. 1. **c** Variable malate (mal) concentrations (mM) were added during the progress of H_2O_2 production induced by G/P and 300 μM succinate. The amount of mitochondria used was 0.5 mg/ml. Traces are representative of duplicate traces from at least three independent experiments

higher reduction state of the carriers was increasing the turnover rate of unaffected CCO molecules (Palacios-Callender et al. 2007). In parallel with the reduction of cytochrome c, a hyperproduction of ROS was described (Palacios-Callender et al. 2004). Similarly CO production by heme oxygenase is known to inhibit CCO and activate ROS production (Zuckerbraun et al. 2007). The mechanisms involved are however not clear.

We hypothesized that the succinate induced H_2O_2 generation by Cpl I as described in this study could be involved in this process. To test this we mimicked the effect of NO (and CO) by treating mitochondria with limited concentrations of cyanide (CN^-) to determine a partial inhibition of CCO. The effect of increasing CN^- on H_2O_2 production of mitochondria treated with G/P and 300 μM succinate is reported in Fig. 3a. Very low increments of CN^- increased progressively the H_2O_2 release (starting at 1.5 μM CN^-). The H_2O_2 production rate increased sharply till 30 μM cyanide and progressed slowly at higher CN^- concentrations. This behaviour closely parallels the inhibition of oxygen consumption by CN^- (Fig. 3b). The effect of low CN^- on H_2O_2 production was visible in conditions of very limited inhibition of respiration. This behaviour is similar to the effect of NO which at low concentrations induces the reduction of cytochrome c before inhibiting significantly O_2 consumption (Palacios-Callender et al. 2007).

We performed a succ→H_2O_2 titration curve with CN^- (30 μM) and compared it to the no CN^- situation (Fig. 3c). The CN^- treatment moved to the left, i.e. to lower succinate concentrations, the succinate titration curve. The Vmax of H_2O_2 production was apparently little affected. Also, the H_2O_2 production was increased at the zero succinate point.

The effect of malate (OAA?) and of CN^- on the succinate induced H_2O_2 production are opposite. The two effectors were shown to act contemporarily in mitochondria, where the CN^--induced increase of H_2O_2 generation was inhibited by malate (Fig. 4). We demonstrated recently that the succ→H_2O_2 process is inhibited by low concentrations of some propargylamines (clorgyline and deprenyl were the most effective) (Zoccarato et al. 2008). Clorgyline did inhibit also the CN^--increased production of H_2O_2 (Fig. 4) although slightly less efficently. It may be

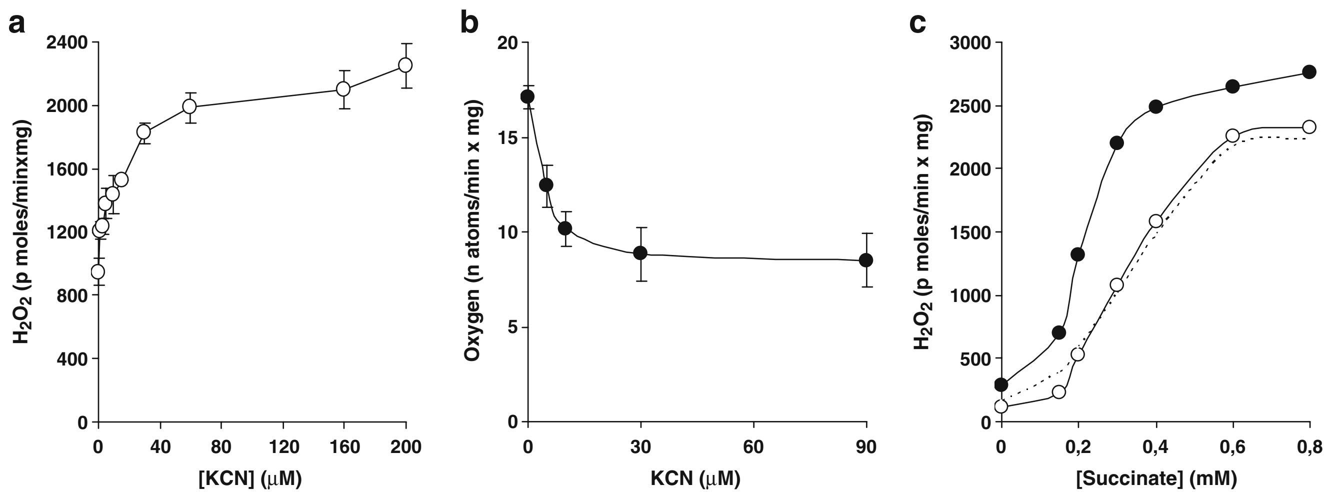

Fig. 3 Positive modulation by KCN of the succinate induced H_2O_2 production. **a** Effect of increasing KCN on the release of H_2O_2 induced by G/P and 300 μM succinate. Values are the means ± S.D. from three experiments. **b** KCN inhibition of respiration in G/P plus 300 μM succinate treated mitochondria. Experimental conditions as in A. Values are the means ± S.D. from at least two independent experiments. **c** Succinate titration of H_2O_2 production in G/P treated mitochondria, in the absence (o) and presence (●) of 30 μM KCN. The points are taken from the same mitochondrial preparation.The experiment was repeated twice. The dotted line reports the values in Fig. 1

concluded that malate and CN^- behave as opposite modulators of the same peroxide generating system.

The H_2O_2 generation in the absence and presence of CN^- is unaffected by the addition of SOD (not shown), a property that was taken as an indicator that O_2^- is produced intramitochondrially (St-Pierre et al. 2002; Zoccarato et al. 2004). The control by the redox state of cytochrome c (and possibly of cytochrome c_1 and the Rieske FeS center of Complex III, together with CCO) on the succ→H_2O_2 release by Cpl I requires an explanation. In the discussion we will give a possible rationale for this behaviour.

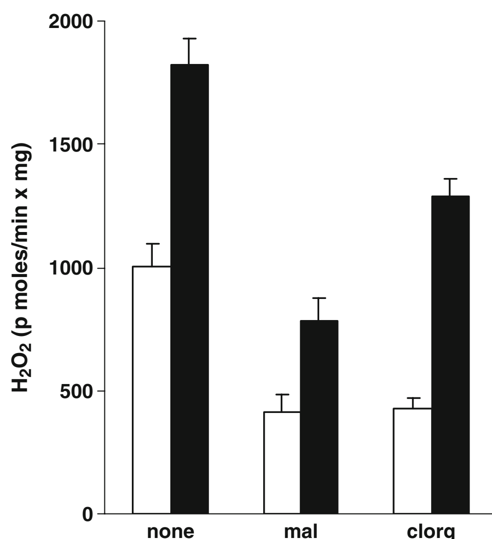

Fig. 4 Malate and clorgyline modulate H_2O_2 generation in the absence and presence of KCN. Mitochondria were incubated with G/P and 300 μM succinate. Both malate (mal, 1 mM) and clorgyline (clorg, 5 μM) decreased H_2O_2 production in the absence (white bars) and presence (black bars) of KCN (30 μM). Data are means ± S.D. from at least two independent experiments

Discussion

The experiments described in this study demonstrate that the mitochondrial concentration of succinate determines the rate of O_2^-/H_2O_2 production by mitochondrial Complex I. A sigmoidal increase of H_2O_2 production was obtained, which started at very low (15 μM) succinate and reached completion at about 600 μM. The semimaximal effect was at about 290 μM. Succinate was effective in the presence of high concentrations of NAD-dependent substrates. The latter substrates determined a low rate of H_2O_2 generation and the addition of succinate activated immediately the stimulated rate. No variations in the mitochondrial membrane potential or in the NADH/NAD ratio were observable upon succinate addition (not shown). We have reported previously that electrons continue to flow down Complex I also in the presence of succinate, i.e. there is no net reverse electron flow. These are presumably the conditions of substrate distribution present *in vivo*. Few data are available on the concentrations of succinate *in vivo*. They range from less than 0.5 mM in heart (Lewandowski et al. 1996) to less than 0.3 mM in brain tissue (Brockmann et al. 2002) to 120 μM in cell cultures (Selak et al. 2005). In kidney a low concentration of 10 μM has been reported recently (Toma et al. 2008). It is important to note that succinate concentration increases sharply in the hypoxic or anoxic state (Folbergrovă et al. 1974; Hoyer and Krier 1986), which

makes it difficult to "freeze" the tissue fast enough to monitor accurately the situation *in vivo*.

The effect of a given succinate concentration on H_2O_2 production is decreased by the presence of another TCA cycle substrate, malate. A fixed malate concentration moves to the right (i.e. to higher succinate concentrations) the succ→H_2O_2 titration curve. The effect of H_2O_2 release inhibition by malate is immediate also at low malate concentrations. It is presently not clear if the effect is due to malate itself or to OAA (a strong competitive inhibitor of succinate oxidation) formed from malate, or to the competition between succinate and malate for the dicarboxylate carrier. The rate of respiration of G/P plus succinate supplied mitochondria was slightly decreased by malate. This may indicate the formation of some OAA. However the rapidity and the extent of the malate effect on H_2O_2 production is remarkable.

A second controller of the succinate induced H_2O_2 release has been identified. This is the reduction level of the respiratory chain carriers on the substrate side of CCO which is increased by the addition of limiting concentrations of the CCO inhibitor CN^-. CN^- increases the succ→H_2O_2 production rate in a graded fashion starting at a very low concentration (1.5 µM) which affects respiration very little. The stimulation of H_2O_2 production closely parallels the degree of inhibition of the mitochondrial oxygen consumption. CN^- moves to the left (to lower succinate concentrations) the succ → H_2O_2 production curve. The CN^- induced increase of H_2O_2 release is evident also at zero succinate. It was reported that the carrier becoming reduced first, at lower levels of CCO inhibition by NO, is cytochrome c (Palacios-Callender et al. 2007). CN^- was used to mimic the effect of NO, which is produced in the cytosol and mitochondria of many cells and which has been shown to be an inhibitor of CCO at physiological concentrations. This effect of NO is known to be accompanied by an increase of ROS production (Palacios-Callender et al. 2004; Quintero et al. 2006a). A similar increase of reactive oxygen species has been shown to be activated in the presence of CO, another inhibitor of CCO, produced by heme oxygenase (Zuckerbraun et al. 2007). So it appears that graded levels of CCO inhibition are physiologically induced, and are accompanied by the mitochondrial generation of increased levels of O_2^-/H_2O_2, under the control of succinate concentration. This increased H_2O_2 production is apparently necessary for the activation of HIF (Murphy 2009),NF-kB (Palacios-Callender et al. 2004) and AMPK (Quintero et al. 2006a). In pathological situations, anoxia will likely lead to the same state of hyper production of ROS, and also in tumors, which are generally hypoxic, a ROS hyperproduction has been described (Quintero et al. 2006b). It is to note that a state of hypoxia, determined by a vessel occlusion, or the NO hyperproduction determined by the induction of inducible nitric oxide synthase during inflammation, will promote an increase of succinate concentration which will further increase the succinate induced H_2O_2 production (Folbergrovă et al. 1974; Hoyer and Krier 1986).

The state of hyperglycemia has been recently identified as another condition leading to accumulation of increased succinate (Toma et al. 2008). Hyperglycemia has indeed been shown to induce an increased generation of H_2O_2 (Nishikawa et al. 2000). Mitochondria have been recognized as responsible for peroxide release, based on the sensitivity to the succinate oxidation inhibitor thenoyltrifluoroacetone and to uncouplers. Most of the complications in diabetes have been linked to increased H_2O_2 release (Brownlee 2005).

Calorie restriction (CR) which is known to increase life span and to decrease cancer, was reported to lead to a decreased activity of the first half of the TCA cycle enzymes, with a decrease of citrate, glutamate and α-ketoglutarate concentrations and an increased malate concentration. Succinate was not measured, but its decrease is conceivable (Hagopian et al. 2004). These properties are compatible with a decreased H_2O_2 production in CR, linked to changes in TCA cycle metabolites.

Data are accumulating showing that in laboratory animals the baseline inflammation level increases with age and that such inflammation (represented by TNF-α, IL-1β, IL-6, COX-2, C-reactive protein etc) decreases with CR (Chung et al. 2009). It may be hypothesized that different levels of stress induce different responses by the succ→H_2O_2 system and that a generalized variation in [succinate] may modulate aging (CR→< [succinate]→< H_2O_2 →>longevity). Two studies report that in mitochondria from CR animals a decreased H_2O_2 production could be evidenced (Gredilla et al. 2001; López-Torres et al. 2002) accompanied by a specific decrease of the oxidative damage to mitochondrial DNA. This may signify that in isolated mitochondria from CR rats the H_2O_2 response to *added* succinate may be decreased, indicating that mitochondria have become primed to perform a lower H_2O_2 response to a given succinate concentration. On the contrary, a higher H_2O_2 response to a given succinate concentration can develop during ageing, or secondary to an inflammatory stress. It can be hypothesized that a stress dependent influx in the mitochondrial intermembrane space of the phosphorylated protein $p66^{shc}$ (Pinton et al. 2007) (whose absence increases by 30% the life span (Migliaccio et al. 1999)) or of some other protein with similar function may modulate the succinate sensitivity of the H_2O_2 release. $p66^{shc}$ was shown to bind to cytochrome c and to activate H_2O_2 release (Giorgio et al. 2005). As shown in this study (experiments with CN^-) reduced cytochrome c may be the species involved in increasing the succ→H_2O_2 response.

It is interesting that some propargylamine derived compounds have been shown to decrease the succinate induced H_2O_2 release (Zoccarato et al. 2008) also in the presence of CN^- (this study). These compounds appear to behave specifically affecting only H_2O_2 production. They could serve as the basis for the synthesis of pharmacologically useful H_2O_2 production inhibitors.

Succinate oxidation-induced increase of O_2^-/H_2O_2 production, also in the presence of high NAD dependent substrates oxidation, in conditions where only foreward electron flux in Cpl I is present is puzzling. No variations in membrane potential or of the NAD/NADH ratio are detectable (on top of what is induced by G/P) in the presence of succinate (see also Lambert et al. 2008). It was suggested that a higher reduction state of the QH_2/Q redox couple is induced by succinate (Murphy 2009; Zoccarato et al. 2007; Lambert et al. 2008). Furthermore, the reduction of electron carriers within Cpl II may be important (Zoccarato et al. 2007, and this study). The properties of the succ→H_2O_2 and its regulation as depicted in this study clearly indicate a functional significance of mitochondrial O_2^-/H_2O_2 production. We propose that succinate specifically activates electron transfer from some electron carrier in Cpl I to O_2 (forming O_2^-) in turn controlled negatively by malate (OAA?) and positively by the redox state of cytochrome c. Such electron transfer may be activated by some succinate promoted interaction between Cpl I and Cpl II. The supercomplex which is known to be formed between Cpl I and Cpl III (Lenaz and Genova 2007); and the interaction between Cpl I, Cpl II, Cpl III and Cpl IV, described recently (Acín-Pérez et al. 2008) may mediate these effects. In particular the interaction between Cpl I and Cpl II may be promoted by succinate oxidation.

Acknowledgements This work was supported by "Finanziamenti di Ateneo", ex 60% funds. We wish to thank Patrizia Pagliarin for skilful technical assistance.

References

Acín-Pérez R, Fernández-Silva P, Peleato ML, Pérez-Martos A, Enriquez JA (2008) Mol Cell 32:529–539
Beltrán B, Quintero M, Garcia-Zaragozá E, O'Connor E, Esplugues JV, Moncada S (2002) Proc Natl Acad Sci USA 99:8892–8897
Bortolami S, Comelato E, Zoccarato F, Alexandre A, Cavallini L (2008) J Bioenerg Biomembr 40:9–18
Brandt U (2006) Annu Rev Biochem 75:69–92
Brockmann K, Bjornstad A, Dechent P, Korenke CG, Smeitink J, Frans Trijbels JM, Athanassopoulos S, Villagran R, Skjeldal OH, Wilichowski E, Frahm J, Hanefeld F (2002) Ann Neurol 52:38–46
Brownlee M (2005) Diabetes 54:1615–1625
Chung HY, Cesari M, Anton S, Marzetti E, Giovannini S, Seo AY, Carter C, Yu BP, Leeuwenburg C (2009) Ageing Res Rev 8:18–30
Folbergrovă J, Ljunggren B, Norberg K, Siesjö BK (1974) Brain Res 80:265–279
Giorgio M, Migliaccio E, Orsini F, Paolucci D, Moroni M, Contursi C, Pelliccia G, Luzi L, Minucci S, Marcaccio M, Pinton P, Rizzuto R, Bernardi P, Paolucci F, Pelicci PG (2005) Cell 122:221–233
Gredilla R, Sanz A, Lopez-Torres M, Barja G (2001) FASEB J 15:1589–1591
Hagopian K, Ramsey JJ, Weindruch R (2004) Exp Gerontol 39:1145–1154
Hoyer S, Krier C (1986) Neurobiol Aging 7:23–29
Huang LS, Shen JT, Wang AC, Berry EA (2006) Bichem Biophys Acta 1757:1073–1083
Kearney EB, Ackrell BAC, Mayr M (1972) Biochem Biophys Res Comm 49:1115–1121
Lambert AJ, Buckingham JA, Brand MD (2008) FEBS Lett 582:1711–1714
Lenaz G, Genova ML (2007) Am J Physiol Cell Physiol 292:C1221–C1239
Lewandowski ED, Doumen C, White LT, LaNoue KF, Damico LA, Yu X (1996) Magn Reson Med 35:149–154
López-Torres M, Gredilla R, Sanz A, Barja G (2002) Free Radic Biol Med 32:882–889
Migliaccio E, Giorgio M, Mele S, Pelicci G, Reboldi P, Pandolfi PP, Lanfrancone L, Pelicci PG (1999) Nature 402:309–313
Muller FL, Liu Y, Abdul-Ghani MA, Lustgarten MS, Bhattacharya A, Jang YC, Van Remmen H (2008) Biochem J 409:491–499
Murphy MP (2009) Biochem J 417:1–13
Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, Brownlee M (2000) Nature 404:787–790
Palacios-Callender M, Quintero M, Hollis VS, Springett RJ, Moncada S (2004) Proc Natl Acad Sci USA 101:7630–7635
Palacios-Callender M, Hollis V, Frakich N, Mateo J, Moncada S (2007) J Cell Sci 120:160–165
Pinton P, Rimessi A, Marchi S, Orsini F, Migliaccio E, Giorgio M, Contursi C, Minucci S, Mantovani F, Wieckowski MR, Del Sal G, Pelicci PG, Rizzuto R (2007) Science 315:659–663
Quintero M, Colombo SL, Godfrey A, Moncada S (2006a) Proc Natl Acad Sci USA 103:5379–5384
Quintero M, Brennan PA, Thomas GJ, Moncada S (2006b) Cancer Res 66:770–774
Selak MA, Armour SM, MacKenzie ED, Boulahbel H, Watson DG, Mansfield KD, Pan Y, Celeste Simon M, Thompson CB, Gottlieb E (2005) Cancer Cell 7:77–85
Starkov AA (2008) Ann NY Acad Sci 1147:37–52
St-Pierre J, Buckingham JA, Roebuck SJ, Brand MD (2002) J Biol Chem 277:44784–44790
Toma I, Kang JJ, Sipos A, Vargas S, Bansal E, Hanner F, Meer E, Peti-Peterdi J (2008) J Clin Invest 118:2526–2534
Turrens JF (2003) J Physiol 552:335–344
Zoccarato F, Cavallini L, Alexandre A (2004) J Biol Chem 279:4166–4174
Zoccarato F, Toscano P, Alexandre A (2005) J Biol Chem 280:15587–15594
Zoccarato F, Cavallini L, Bortolami S, Alexandre A (2007) Biochem J 406:125–129
Zoccarato F, Cappellotto M, Alexandre A (2008) J Bioenerg Biomembr 40:289–296
Zuckerbraun BS, Chin BY, Bilban M, de Costa d'Avila J, Rao J, Billiar TR, Otterbein LE (2007) FASEB J 21:1099–1106