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Generation of reactive oxygen species by the mitochondrial electron transport chain

Yuanbin Liu,* Gary Fiskum† and David Schubert*

*Cellular Neurobiology Laboratory, The Salk Institute for Biological Studies, La Jolla, California, USA

†Department of Anesthesiology, University of Maryland School of Medicine, Baltimore, Maryland, USA

Abstract

Generation of reactive oxygen species (ROS) by the mitochondrial electron transport chain (ETC), which is composed of four multiprotein complexes named complex I–IV, is believed to be important in the aging process and in the pathogenesis of neurodegenerative diseases such as Parkinson's disease. Previous studies have identified the ubiquinone of complex III and an unknown component of complex I as the major sites of ROS generation. Here we show that the physiologically relevant ROS generation supported by the complex II substrate succinate occurs at the flavin mononucleotide group (FMN) of complex I through reversed electron transfer, not at the

ubiquinone of complex III as commonly believed. Indirect evidence indicates that the unknown ROS-generating site within complex I is also likely to be the FMN group. It is therefore suggested that the major physiologically and pathologically relevant ROS-generating site in mitochondria is limited to the FMN group of complex I. These new insights clarify an elusive target for intervening mitochondrial ROS-related processes or diseases.

Keywords: aging, electron transport chain, mitochondria, Parkinson's disease, reactive oxygen species.

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The mitochondrial electron transport chain (ETC) has been recognized as one of the major cellular generators of reactive oxygen species (ROS), which include superoxide (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl free radical ($\cdot OH$; Loschen *et al.* 1971; Boveris *et al.* 1972; Chance *et al.* 1979). It was found that some of the electrons passing through the mitochondrial ETC leak out to molecular oxygen (O_2) to form superoxide which is quickly dismutated by the mitochondrial superoxide dismutase (Mn-SOD) to H_2O_2 (Loschen *et al.* 1974; Boveris and Cadenas 1975). The production of ROS by mitochondria is believed to be important in the aging process and in the pathogenesis of neurodegenerative diseases such as Parkinson's disease (Shigenaga *et al.* 1994; Wallace 1999; Betarbet *et al.* 2000). Recent Mn-SOD knockout mice experiments (Li *et al.* 1995; Lebovitz *et al.* 1996) clearly demonstrate that mitochondrial ROS generation is a physiologically significant process *in vivo*, and that mitochondrial SOD is essential for maintaining the normal function of mitochondria-rich organs such as heart, brain, and liver. However, it remains a challenge to define the physiologically or pathologically relevant sites of ROS formation in the mitochondrial ETC and to find clinically useful agents that can minimize mitochondrial ROS production.

The mitochondrial ETC is composed of a series of electron carriers (flavoproteins, iron-sulfur proteins, ubiquinone and cytochromes) with standard redox potentials (E_o') ranging from -0.320 to $+0.380$ V (Wilson *et al.* 1974). These carriers are arranged spatially according to their redox potentials and organized into four complexes (Fig. 1). Electrons derived from metabolic reducing equivalents (NADH and $FADH_2$) are fed into the ETC through either complex I or complex II, and eventually pass to molecular oxygen (O_2 , $E_o' = +0.815$ V) to form H_2O in complex IV. Thermodynamically, all of these electron carriers in their reduced form could pass their electron to O_2 to form superoxide. However, previous studies

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Address correspondence and reprint requests to Y. Liu, Cellular Neurobiology Laboratory, The Salk Institute for Biological Studies, 10010 N. Torrey Pines Road, La Jolla, CA 92037-9062, USA.

E-mail: liu@salk.edu

Abbreviations used: CDNB, 1-chloro-2,4-dinitrobenzene; DPI, diphenyleneiodonium; ETC, electron transport chain; FAD, flavin adenine dinucleotide; FCCP, carbonyl cyanide p-trifluoromethoxyphenylhydrazone; FMN, flavin mononucleotide; GSH, glutathione; HRP, horseradish peroxidase; p-HPAA, p-hydroxyphenylacetic acid; ROS, reactive oxygen species; SOD, superoxide dismutase.

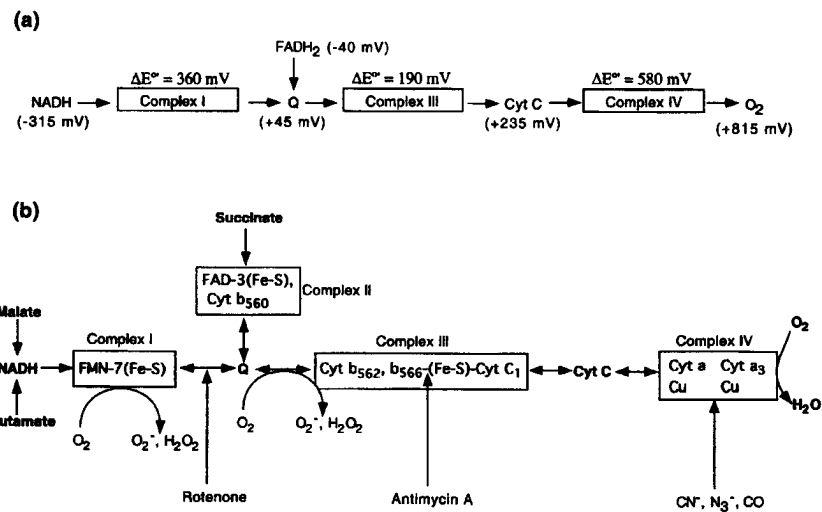


Fig. 1 The mitochondrial electron transport chain. (a) The standard reduction potentials (E°) of its most mobile components and the ΔE° 's of three complexes where sufficient free energy is harvested to synthesize ATP. (b) The sites of ROS production and the sites of action of commonly used respiratory inhibitors.

with isolated mitochondria detected only two ROS-forming sites in the mitochondrial ETC, namely the ubiquinone site in complex III and an unknown site in complex I (Turrens and Boveris 1980; Turrens *et al.* 1985; Cadenas *et al.* 1977, see also Fig. 1b). The physiological or pathological relevance of the ubiquinone site in complex III is controversial because ROS production at this site is usually artificially induced with the complex III inhibitor anti-mycin A (Forman and Azzi 1997). There are also considerable variations in the capacity to generate ROS in terms of rates and substrate specificity by mitochondria isolated from different tissues and species (Boveris *et al.* 1972; Cino and Del Maestro 1989; Kwong and Sohal 1998). These variations, although not understood at present, do suggest that the generation of ROS by the mitochondria ETC could be modified.

We have reexamined the generation of ROS by the mitochondrial ETC. The major new finding of the study is that the primary physiologically and pathologically relevant site of ROS generation in the mitochondrial ETC is the flavin mononucleotide (FMN) group of complex I, not the ubiquinone of complex III as previously thought. These novel insights have important implications for developing drugs that may delay aging and ameliorate mitochondrial ROS-related diseases.

Materials and methods

Preparation of mitochondria and submitochondrial particles

Rat brain was removed under anesthesia and placed in ice-cold buffer containing 225 mM mannitol, 75 mM sucrose, 1 mM ethyleneglycol-bis-(β -aminoethyl) N,N,N',N'-tetraacetic acid (EGTA), 5 mM K-4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.4 and 1 mg/mL bovine serum albumin (BSA; MSE buffer). The tissue was minced, washed with MSE and homogenized in 10 mL MSE containing 5 mg Nagase. The volume of the homogenate was brought to 30 mL with MSE and centrifuged at 2000 g

for 3 min. The supernatant was centrifuged at 12 000 g for 8 min. The resulting pellet, containing free mitochondria and synaptosomes, was resuspended in MSE containing 0.02% digitonin to disrupt synaptosomal plasma membrane and release any mitochondria trapped within, and then centrifuged at 12 000 g for 10 min. The pellet was resuspended in MSE medium containing EGTA. It is important to resuspend mitochondria in a buffer containing EGTA because even the contaminating amount of calcium in the buffer will severely inhibit mitochondrial ROS production. Mitochondrial protein concentration was by determined by a modified biuret assay using BSA as standard.

Submitochondrial particles derived from rat brain mitochondria were prepared according to Cino and Del Maestro (1989). Rat heart mitochondria and rat liver mitochondria were isolated according to Tyler and Gonze (1967) and Holland *et al.* (1973), respectively.

Measurement of mitochondrial H_2O_2 release

Mitochondrial generation of H_2O_2 was measured by monitoring H_2O_2 -induced fluorescence of p-hydroxyphenylacetic acid (p-HPAA) ($\lambda_{\text{ex}} = 317 \text{ nm}$, $\lambda_{\text{em}} = 414 \text{ nm}$) under the catalysis of horseradish peroxidase (HRP; Guilbaut *et al.* 1968; Liu *et al.* 1993; Tan *et al.* 1998). Mitochondria (0.1 mg protein/mL) were incubated at 37°C in the KCl buffer (125 mM KCl, 2 mM K_2HPO_4 , 1 mM MgCl_2 and 20 mM HEPES, pH 7.4) plus the H_2O_2 -detection system (0.2 mM p-HPAA and 0.5 μM HRP). Either 6 mM succinate or 6 mM malate plus 6 mM glutamate were added to initiate H_2O_2 production. A known amount of H_2O_2 was added to the cuvette for the quantitation of the fluorescence signal, which was detected by a Perkin-Elmer LS-50B fluorescence spectrometer with temperature control and a magnetic stirring device. The efficiency of the H_2O_2 -detection method was assessed by measuring the rate of oxygen consumption and H_2O_2 generation of a glucose/glucose oxidase system. The detection efficiency was 60% for 0.2 mM p-HPAA plus 0.5 μM HRP, and 75% for 1 mM p-HPAA plus 0.5 μM HRP. The effect of p-HPAA on mitochondrial function was assessed by mitochondrial oxygen consumption. The optimal mitochondria concentration was also studied to assure a linear H_2O_2 release rate. Mitochondria, 0.1 mg/mL, in the presence of 0.2 mM p-HPAA plus 0.5 μM HRP always give a linear H_2O_2 release rate, was therefore used for all the

experiments in this study. Mitochondrial H_2O_2 release was also studied in a chloride ion-free medium (0.25 M mannitol, 75 mM sucrose, 5 mM HEPES, 2.5 mM potassium phosphate, pH 7.4, 5 mM $MgSO_4$, 1 mM EGTA).

Measurement of mitochondrial NAD(P)H level with pyridine nucleotide fluorescence

The level of mitochondrial NAD(P)H was monitored by recording their relative fluorescence intensity ($\lambda_{ex} = 352$ nm, $\lambda_{em} = 464$ nm) at 37°C with a Perkin-Elmer LS-50B fluorescence spectrometer. Mitochondria were suspended at 0.3 mg/mL in a sucrose-containing medium (0.25 M mannitol, 75 mM sucrose, 5 mM HEPES, 2.5 mM potassium phosphate, pH 7.4, 5 mM $MgSO_4$, 1 mM EGTA) to archive the required signal intensity.

Measurement of superoxide production by submitochondrial particles

Superoxide was measured by the SOD-inhibitable oxidation of adrenaline to adrenochrome according to Boveris (1984). The assay medium (pH 7.4) contained 1 mM adrenaline, 0.5 μ M catalase, 1 mg/mL SMP. Superoxide production was initiated with 50 μ M NADH plus 1 μ M rotenone or 6 mM succinate plus 1 μ M anti-mycin A. Formation of adrenochrome was followed at 480 nm ($E = 4.0$ mm/cm) with a Shimadzu UV160 dual-beam spectrophotometer equipped with temperature control and magnetic stirring devices.

Oxygen consumption

Oxygen consumption by isolated mitochondria (0.1 mg/mL) in the chloride-free medium was measured polarographically with a Clark type oxygen electrode in a thermostatically controlled micro chamber (Instech, Plymouth Meeting, PA, USA) equipped with a magnetic stirring device. The solubility of oxygen in air-saturated medium at 37°C was taken to be 390 ng-atoms/mL.

Results and Discussion

H_2O_2 is commonly used as a measure for mitochondrial ROS production because superoxide produced by the mitochon-

drial ETC is rapidly converted to H_2O_2 by Mn-SOD and consequently is not detectable (Forman and Azzi 1997). H_2O_2 readily penetrates membranes and can be used as a stoichiometric indicator of mitochondrial superoxide production (Loschen *et al.* 1974; Boveris and Cadenas 1975). We have previously found that HRP plus the fluorescent substrate p-HPAA constitutes a sensitive and specific method for quantifying mitochondrial H_2O_2 release (Liu *et al.* 1993; Tan *et al.* 1998). We studied H_2O_2 production by rat mitochondria with this method and have confirmed most of the results of previous studies (Loschen *et al.* 1971; Boveris *et al.* 1972; Chance *et al.* 1979; Cino and Del Maestro 1989; Kwong and Sohal 1998). For example, the FAD-linked substrate succinate supports the highest rate of H_2O_2 production in the absence of respiratory inhibitors (Figs 2a–c). Succinate-supported H_2O_2 production is greatest during state 4 respiration (oxygen consumption in the absence of ADP), but was abolished by ADP (state 3 respiration, Table 1) and by carbonyl cyanide p-trifluoromethoxyphenylhydrozone (FCCP, uncoupled state respiration; Table 1). H_2O_2 release supported by NADH-linked substrates such as malate/glutamate is not detectable with rat brain mitochondria. It only becomes detectable in the presence of the complex I inhibitor rotenone or the complex III inhibitor anti-mycin A (Table 1). Rat liver mitochondria supported by malate/glutamate do, however, produce detectable H_2O_2 in the absence of respiratory inhibitors (Fig. 2d) as have been reported before (Boveris *et al.* 1972).

Contrary to the current prevalent view that most succinate-supported ROS production occurs at the ubiquinone site of complex III (Turrens *et al.* 1985; Cadenas *et al.* 1977), the observation that succinate-supported H_2O_2 production by rat brain mitochondria is almost totally abolished by the specific complex I inhibitor, rotenone, led Cino and Del Maestro (1989) to suggest that succinate-supported H_2O_2 production must be occurring within complex I through a reversed

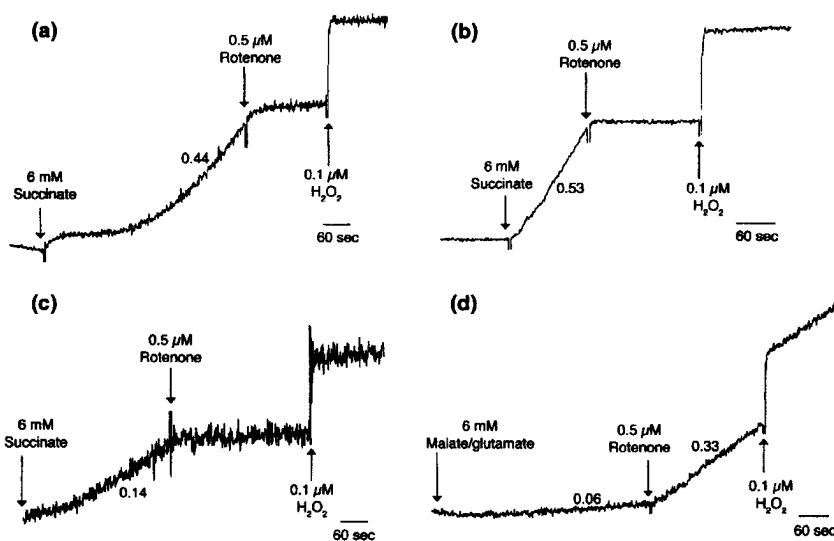


Fig. 2 H_2O_2 release by mitochondria isolated from various rat tissues: (a) brain mitochondria, (b) heart mitochondria, (c and d) liver mitochondria. Y-axis is relative fluorescence and X-axis is time in s. Numbers along the traces are in nMol H_2O_2 /min/mg protein.

Table 1 Generation of H₂O₂ by isolated rat brain mitochondria: effects of substrates and inhibitors

Addition	Mitochondrial H ₂ O ₂ release (nmol/min/mg protein)
Succinate	0.39 ± 0.03 ^a
Succinate + 0.1 mM ADP	0.03 ± 0.01
Succinate + 0.5 μM FCCP	0
Succinate + 0.5 μM rotenone	0
Succinate + 6 mM malonate	0
Succinate + 0.5 μM anti-mycin A	0.71 ± 0.03
Succinate + 0.5 μM anti-mycin A + 0.1 mM ADP	0.69 ± 0.03
Succinate + 0.5 μM anti-mycin A + 0.5 μM rotenone	0.70 ± 0.03
Succinate + 0.5 μM anti-mycin A + 0.5 μM FCCP	0.74 ± 0.04
Succinate + 1 μM DPI	0.03 ± 0.01
Succinate + 60 μM CDNB	1.71 ± 0.06
Succinate + 60 μM CDNB + 0.5 μM rotenone	0.01 ± 0.01
Succinate + 60 μM CDNB + 0.5 μM anti-mycin A	0.74 ± 0.03
Malate/glutamate	0
Malate/glutamate + 0.5 μM rotenone	0.25 ± 0.02
Malate/glutamate + 0.5 μM rotenone + 1 μM DPI	0.08 ± 0.01
Malate/glutamate + 60 μM CDNB	0.45 ± 0.04
Malate/glutamate + 60 μM CDNB + 0.5 μM rotenone	0.56 ± 0.05

^aData are mean ± SEM of three experiments. FCCP, carbonyl cyanide p-trifluoromethoxyphenylhydrazone; DPI, diphenyleneiodonium; CDNB, 1-chloro-2,4-dinitrobenzene.

electron transfer mechanism (from succinate to complex I, see Fig. 1b). We now show that the blockage of succinate-supported H₂O₂ production by rotenone is a general phenomenon since it is observed not only with rat brain mitochondria (Fig. 2a and Table 1), but also with mitochondria from rat heart (Fig. 2b), rat liver (Fig. 2c), and dog brain (Liu *et al.* 1993). Votyakova and Reynolds (2001) recently confirm once again that succinate-supported H₂O₂ production by rat brain mitochondria is largely blocked by rotenone.

Reversed electron transfer refers to the phenomenon that in the absence of ADP electrons derived from succinate can reversely flow to complex I and reduce NAD⁺ to NADH (Chance and Hollunger 1961a,b; Hinkle *et al.* 1967). Reversed electron transfer is inhibited by the agents that deplete the mitochondrial proton gradient (e.g. ADP and FCCP). The pathway of electron transfer from succinate to NAD⁺ appears to include electron carriers up to the anti-mycin A-sensitive site in complex III because anti-mycin A blocks reversed electron transfer (Chance and Hollunger 1961b; Hinkle *et al.* 1967). We have confirmed these observations with rat brain mitochondria and demonstrated a tight link between succinate-supported reversed electron transfer and ROS production. As shown in Fig. 3(a), succinate supports rapid reduction of NAD⁺ as indicated by the increased NADH fluorescence. Addition of the specific succinate dehydrogenase (complex II) inhibitor

malonate completely abolished the reduction of NAD⁺ by succinate, suggesting that the electrons for the reduction of NAD⁺ come directly from succinate through reversed electron transfer, not from succinate converted NADH-generating substrates such as malate. Figure 3(b and c) show that ADP, FCCP and anti-mycin A all inhibit or block succinate-supported NADH formation as reported before (Chance and Hollunger 1961b; Hinkle *et al.* 1967). A tight link between succinate-supported reversed electron transfer and ROS production is evident since blockers of reversed electron transfer (e.g. ADP, FCCP and malonate) also blocks ROS production (Table 1). However, the complex I inhibitor rotenone is not suitable to study this link because its effect on mitochondrial NADH level is complex. On one hand, rotenone blocks succinate-supported NADH formation through reversed electron transfer and therefore should decrease NADH level. On the other hand, it blocks NADH oxidation through complex I. Because succinate will be converted through the tricarboxylic acid cycle to NADH-generating substrates such as malate, blocking complex I can result in increased NADH level. The end result is that rotenone actually increases mitochondrial NADH level with succinate as the substrate (Fig. 3d). Rotenone-induced increase in mitochondrial NADH level is not sensitive to ADP, FCCP, malonate and anti-mycin A (Figs 3a–c).

The current, prevalent view that the ubiquinone site in complex III is responsible for succinate-supported ROS generation derives from the observation that succinate-supported H₂O₂ production in the presence of anti-mycin A occurs at the ubiquinone site in complex III (Turrens *et al.* 1985; Cadenas *et al.* 1977). However, anti-mycin A plus succinate-supported mitochondrial ROS production is not affected by agents (ADP, FCCP and rotenone) that block succinate-supported ROS production (Table 1). Its physiological significance is also not clear since there is no evidence for an *in vivo* factor that acts like anti-mycin A. The physiologically relevant ROS production supported by succinate in state 4 apparently occurs at complex I through reversed electron transfer.

Additional evidence that succinate-supported H₂O₂ production occurs at complex I through reversed electron transfer comes through the use of the glutathione (GSH)-depleting agent, 1-chloro-2,4-dinitrobenzene (CDNB). CDNB has been found to quickly deplete mitochondrial GSH (within 2 min at 60 μM) with very little effect on their respiration and membrane potential (Jocelyn and Cronshaw 1985). *In vivo*, H₂O₂ in mitochondria is scavenged by glutathione peroxidase using GSH as a substrate (Chance *et al.* 1979). The non-renewable, residual GSH in isolated mitochondria, however, is not expected to have a significant effect on the rate of H₂O₂ release for it is rapidly depleted by the steadily generated H₂O₂. Surprisingly, CDNB enhanced succinate-supported H₂O₂ release by over sixfold, which was still blocked by rotenone (Fig. 4a, Table 1), but had no significant effect on

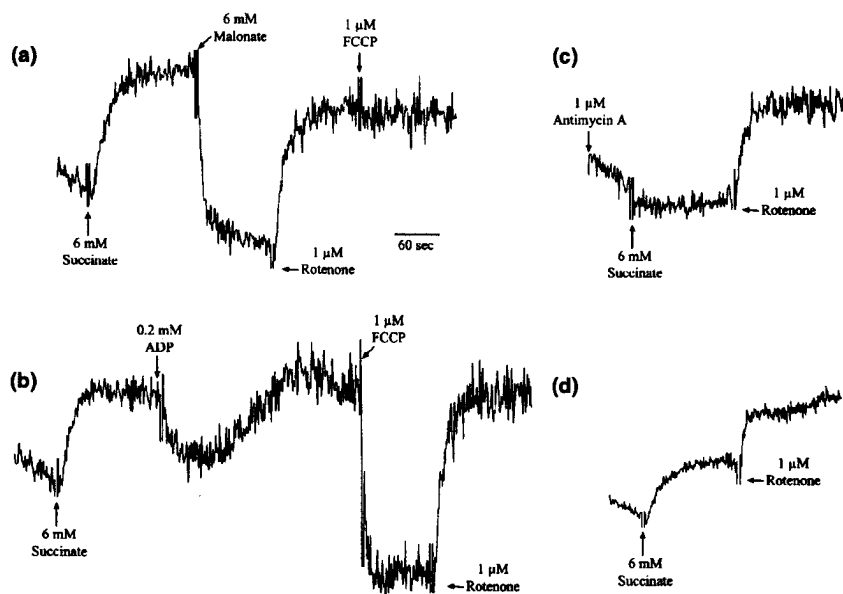


Fig. 3 Succinate-supported NADH formation: effects of various inhibitors. (a) Malonate blocks succinate-supported NADH formation. (b) ADP and FCCP inhibit or block succinate-supported NADH formation. (c) Anti-mycin A blocks succinate-supported NADH formation. (d) Rotenone increases mitochondrial NADH level. Y-axis is relative fluorescence and X-axis is time in s.

H₂O₂ release supported by succinate plus anti-mycin A (Fig. 4b). CDNB also significantly increased H₂O₂ release supported by malate plus glutamate (Table 1). Similar results were also observed by Zoccarato *et al.* (1988) with guinea pig brain mitochondria. It is difficult to explain these effects of CDNB on mitochondrial H₂O₂ release by its GSH-depleting effect. However, these effects can easily be explained if CDNB directly and selectively increases superoxide production by complex I but has no effect on superoxide production by complex III. Rat brain submitochondrial particles, which

are devoid of GSH, were used to test this possibility. Figure 4(c and d) shows that CDNB indeed directly increases superoxide production in complex I (NADH plus rotenone) by fourfold but has no effect on superoxide production by complex III (succinate plus anti-mycin A).

The above results show that the physiologically relevant ROS production supported by succinate occurs in complex I through reversed electron transfer. ROS generation in the presence of anti-mycin A, on the other hand, occurs at the ubiquinone region of complex III (Turrens *et al.* 1985;

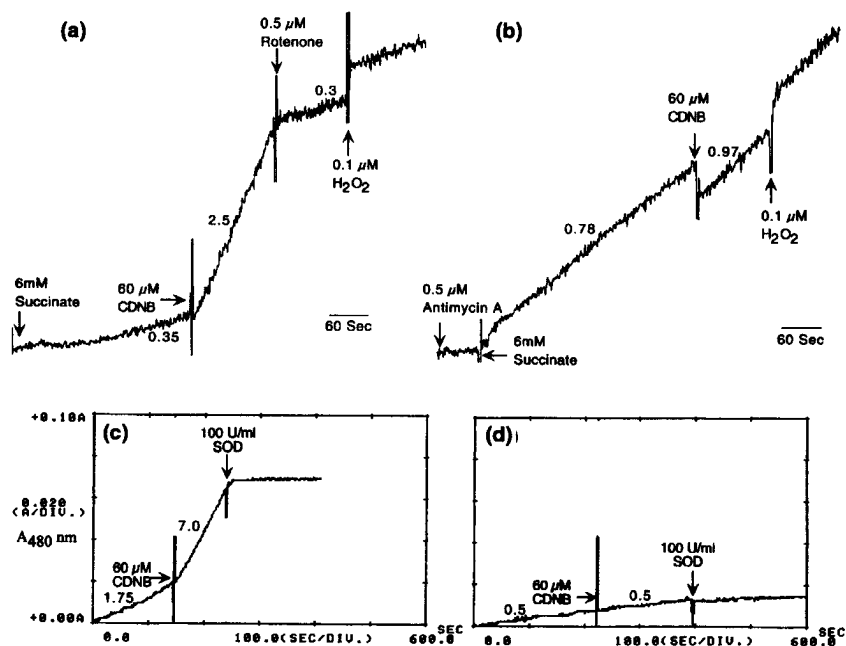


Fig. 4 Effects of CDNB on H₂O₂ release by rat brain mitochondria and on superoxide production by rat brain submitochondrial particles (SMP). (a) CDNB dramatically increases succinate-supported H₂O₂ release. (b) CDNB does not affect H₂O₂ release supported by succinate and anti-mycin A. (c) CDNB enhances NADH (50 μM)/rotenone (1 μM)-supported superoxide production by SMP. (d) CDNB does not affect succinate (6 mM)/anti-mycin A (1 μM)-supported superoxide production by SMP. Numbers along the traces are in nmol H₂O₂/min/mg protein for (a and b) and nmol O₂-(min/mg protein for (c and d).

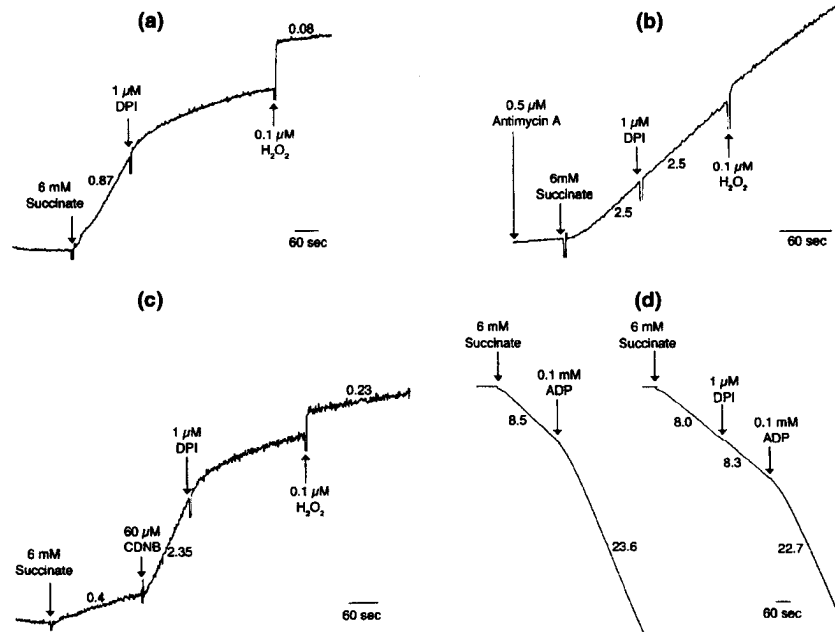


Fig. 5 DPI blocks succinate-supported H₂O₂ release without affecting the activity of complex II. (a) 1 μM DPI inhibits succinate-supported H₂O₂ release. (b) 1 μM DPI does not inhibit succinate/anti-mycin A-supported H₂O₂ release. (c) 1 μM DPI inhibits succinate/CDNB-supported H₂O₂ release. (d) 1 μM DPI does not affect succinate-supported oxygen consumption. All experiments were done in a chloride ion-free medium using rat heart mitochondria. Numbers along the traces are in nmol H₂O₂/min/mg protein for (a to c) and nmol O atom/min/mg protein for (d).

Cadenas *et al.* 1977). While the physiological and pathological significance of anti-mycin A-induced ROS production at complex III is not clear, there is ample evidence that ROS production at complex I is important in the pathogenesis of Parkinson's disease and other mitochondrial diseases related to complex I deficiencies (Robinson 1998; Betarbet *et al.* 2000). It can therefore be argued that the major physiologically and pathologically relevant mitochondrial ROS-generating site is in complex I. The remaining question is which component of complex I is responsible for ROS production?

It has been considered very difficult to identify the exact ROS-forming site within complex I because the FMN group and the 6–7 iron-sulfur centers in complex I all have the potential to form ROS (Wilson *et al.* 1974; Turrens and Boveris 1980). Fortunately, the flavoprotein inhibitor diphenyleneiodonium (DPI) allowed us to deduce that the exact ROS-forming site within complex I is the FMN group. Long before DPI was used as an inhibitor of the neutrophil/macrophage superoxide-generating NADPH oxidase (Cross and Jones 1986), DPI was found to selectively inhibit the activity of complex I, both *in vitro* and *in vivo*, despite the fact that complex II also contains a flavin group (FAD; Holland *et al.* 1973; Ragan and Bloxham 1977). In chloride ion-containing medium, DPI catalyzes a compulsory exchange of chloride ions for hydroxyl ions across the inner mitochondrial membrane, which results in the swelling of mitochondria and the inhibition of both complex I and complex II. However, in chloride ion-free medium, DPI selectively inhibits complex I and does not inhibit succinate oxidation even at 100 μM (Holland *et al.* 1973). Injection of rats with DPI also demonstrated that DPI does not alter chloride ion distribution and that it selectively inhibits

complex I *in vivo* (Holland *et al.* 1973). The rationale for using DPI to identify the exact ROS-forming site within complex I is the following. Because succinate-supported ROS production occurs at complex I through reversed electron transfer, if we can demonstrate that succinate-supported ROS production is blocked by DPI in a condition that does not affect the activity of complex II (e.g. in Cl⁻ ion-free medium), then we can conclude that the ROS-forming site in complex I is the FMN group of the flavoprotein as it is the first electron carrier of complex I, otherwise the ROS-forming site in complex I would be one or more of the iron-sulfur centers. As shown in Fig. 5, 1 μM DPI almost totally inhibits succinate-supported H₂O₂ production in Cl⁻ ion-free medium (Fig. 5a). Two pieces of evidence suggest that the activity of complex II is not affected by DPI under the same conditions. The first is that succinate plus anti-mycin A-supported H₂O₂ production at complex III is not affected by DPI (Fig. 5b). This result also demonstrates once again that succinate-supported H₂O₂ production and succinate plus anti-mycin A-induced H₂O₂ production occur through different mechanisms. The second is that succinate-supported oxygen consumption is not affected by DPI either in state 4 or state 3 (Fig. 5d), while malate/glutamate-supported oxygen consumption in state 3 is inhibited by DPI by more than 50% under the same conditions [9.2 ± 0.5 (without DPI) vs. 4.4 ± 0.2 (with 1 μM DPI) nmol O/min/mg protein, mean ± SEM, n = 3]. Succinate-supported H₂O₂ production in the presence of CDNB is also blocked by DPI (Fig. 5c). These results suggest that succinate-supported ROS production occurs at the FMN group of complex I and that CDNB may increase ROS production by increasing electron leakage from the

FMN group to oxygen. It is not surprising that malate/glutamate-supported H_2O_2 production is also inhibited by DPI as the FMN group is the first electron carrier of complex I (Table 1). Malate/glutamate-supported ROS generation is also likely to occur at the FMN group of complex I as electrons in complex II travel all the way through the iron-sulfur centers but only generate ROS at the FMN group. It is not clear why electrons passing through the iron-sulfur centers are not leaked to oxygen to form ROS. Inaccessibility to oxygen or other constraints could be invoked to explain this observation, but the final answer may have to wait for the crystal structure of complex I.

The identification of the FMN group in complex I as the major physiologically and pathologically relevant ROS-generating site in the mitochondrial ETC provides a pharmacologically accessible target for delaying aging and for treating neurodegenerative diseases such as Parkinson's. Inhibiting ROS production by mitochondria without affecting normal respiration would constitute a novel approach to intervene in mitochondrial ROS-related processes or diseases. The next challenge is to find clinically useful drugs that can minimize mitochondrial ROS production without affecting normal mitochondrial function.

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