Superoxide Activates Uncoupling Proteins by Generating Carbon-centered Radicals and Initiating Lipid Peroxidation

STUDIES USING A MITOCHONDRIA-TARGETED SPIN TRAP DERIVED FROM α-PHENYL-N-tert-BUTYLNITRONE*

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Although the physiological role of uncoupling proteins (UCPs) 2 and 3 is uncertain, their activation by superoxide and by lipid peroxidation products suggest that UCPs are central to the mitochondrial response to reactive oxygen species. We examined whether superoxide and lipid peroxidation products such as 4-hydroxy2-trans-nonenal act independently to activate UCPs, or if they share a common pathway, perhaps by superoxide exposure leading to the formation of lipid peroxidation products. This possibility can be tested by blocking the putative reactive oxygen species cascade with selective antioxidants and then reactivating UCPs with distal cascade components. We synthesized a mitochondria-targeted derivative of the spin trap α-phenyl-N-tert-butylnitrone, which reacts rapidly with carbon-centered radicals but is unreactive with superoxide and lipid peroxidation products. [4-[4-[[1,1-Dimethylethyl]oxidoimino]methyl[phenoxo]butyl]triphenylphosphonium bromide (MitoPBN) prevented the activation of UCPs by superoxide but did not block activation by hydroxynonenal. This was not due to MitoPBN reacting with superoxide or the hydroxyl radical or by acting as a chain-breaking antioxidant. MitoPBN did react with carbon-centered radicals and also prevented lipid peroxidation by the carbon-centered radical generator 2,2'-azobis(2-methyl propionamidine) dihydrochloride (AAPH). Furthermore, AAPH activated UCPs, and this was blocked by MitoPBN. These data suggest that superoxide and lipid peroxidation products share a common pathway for the activation of UCPs. Superoxide releases iron from iron-sulfur center proteins, which then generates carbon-centered radicals that initiate lipid peroxidation, yielding breakdown products that activate UCPs.

The mitochondrial respiratory chain is a major source of superoxide and derived reactive oxygen species (ROS) in vivo (1, 2). Pathological oxidative damage to mitochondria in diseases and aging is a consequence of this ROS production, although there are many protective and adaptive responses to prevent and repair oxidative damage (2, 3).

Recently, it has been shown that the GDP-sensitive proton conductance catalyzed by uncoupling proteins (UCPs) increases on exposure to superoxide (4) or to lipid peroxidation breakdown products such as 4-hydroxy-2-trans-nonenal (HNE) (5). Three UCPs have been characterized in mammals (6). UCP1 increases the proton conductance of the mitochondrial inner membrane in brown adipose tissue (BAT) for thermogenesis; in contrast, the physiological role of its homologs UCP2 and UCP3 is uncertain. One possibility (7) is that UCPs limit the magnitude of the protonotive force, $\Delta$p, and thus decrease mitochondrial ROS production, which increases dramatically at high $\Delta$p (8). The complete dependence of proton conductance through UCP2 and UCP3 on superoxide or HNE (4, 5) suggests an appealing feedback mechanism in which ROS or oxidative damage activate uncoupling by UCPs, lower $\Delta$p, and decrease ROS production (7). That UCP2 and UCP3 have a role in preventing mitochondrial oxidative damage is supported by the increased expression of UCPs in response to elevated mitochondrial oxidative damage (6, 9) and by the observation that oxidative damage is increased in mitochondria from UCP3−/− mice (10, 11).

To investigate the putative antioxidant role of UCPs, it is important to know whether superoxide and lipid peroxidation products interact with UCPs by distinct mechanisms (e.g. alternative allosteric sites on UCPs) or at different points on the
same pathway (e.g. by superoxide exposure leading to the formation of lipid peroxidation products). To distinguish between these possibilities, antioxidants that block different components of the putative oxidative damage cascade leading from superoxide to peroxidation products such as HNE should be informative. Previously we found that the superoxide stimulation of UCPs was blocked by mitochondria-targeted antioxidants (12). These compounds are selectively directed to and accumulate within mitochondria due to their conjugation to the lipophilic triphenylphosphonium cation (13, 14). Targeting of derivatives of ubiquinol (MitoQ) or acetylpherothol (MitoVit E) to mitochondria showed that it was superoxide within the matrix that led to UCP activation (12). However, as both these compounds react with a range of ROS and lipid peroxidation intermediates (14, 15), it was not possible to distinguish between superoxide itself or a downstream product of the ROS cascade as the UCP activator (12).

We have now synthesized a more selective mitochondria-targeted antioxidant, MitoPBN, which is derived from α-phenyl-N-tetra-butyl nitrite (PBN). The spin trap PBN was chosen because of its high reactivity with carbon-centered radicals (16, 17), whereas its reactivity with superoxide and peroxyl radicals is low, and it does not prevent lipid peroxidation by a chain-breaking antioxidant mechanism (18). MitoPBN should be accumulated within mitochondria and there react preferentially with carbon-centered radicals but not with superoxide. Here we show that MitoPBN prevents the activation of proton conductance through UCPs by superoxide but not by HNE. Furthermore, a carbon-centered radical generator stimulates UCPs, and this activation is also blocked by MitoPBN. These data suggest that superoxide and lipid peroxidation products are both components of a single oxidative damage pathway that activates UCPs. This pathway starts with superoxide as the initiator of a cascade of oxidative damage pathway that activates UCPs. This pathway was repeated 9 times. The residue was dissolved in dichloromethane (20 ml) and washed twice with aqueous potassium bromide (15 ml, 20%, pH 7.0). The organic layer was dried (MgSO4) and evaporated in vacuo to a volume of 2 ml. This solution was then added dropwise to ether (100 ml) with brisk stirring. A pale yellow precipitate formed, and once it had settled the solvent was decanted. The resulting precipitate was dissolved in minimal dichloromethane and added dropwise to excess ether with brisk stirring. Once the precipitate had settled, the solvent layer was decanted. This precipitation process was repeated 3 times. The residue was dried in vacuo for 2 h to give 2 as a pale yellow solid (1.00 g, 1.69 mmol, 48%). UV spectroscopy gave a λmax of 305 nm (ε 18,900 m 2 cm −1). The NMR data were as follows: 1H NMR δ 8.26 (2H, d, J = 4.5 Hz, o-H Ar–CH = N), 7.62–7.87 (15H, m, ArH), 7.47 (1H, s, CH = N), 6.85 (2H, d, J = 4.6 Hz, o-H Ar–O–C), 4.15 (2H, t, J = 5.4 Hz, O–CH2–C), 3.85–3.95 (2H, m, p–CH2–C), 2.26 (2H, quintet, J = 6.3 Hz, O–CH2–C) 1.91–1.92 (2H, m, p–CH2– C), 1.60 (9H, s, C–(C3)3) ppm. 13C NMR δ 25.20 ppm. ESMS found (M+) 510.2554 calculated for C33H37O2NP (M+): 510.2556. Octan-1-ol/PBS partition coefficients were determined as described at room temperature (22). The white precipitate was filtered off, and the green solution was evaporated to dryness in vacuo. The residue was dissolved in dichloromethane (20 ml) and washed twice with aqueous potassium bromide (15 ml, 20%, pH 7.0). The organic layer was dried (MgSO4) and evaporated in vacuo to a volume of 2 ml. This solution was then added dropwise to ether (100 ml) with brisk stirring. A pale yellow precipitate formed, and once it had settled the solvent was decanted. The resulting precipitate was dissolved in minimal dichloromethane and added dropwise to excess ether with brisk stirring. Once the precipitate had settled, the solvent layer was decanted. This precipitation process was repeated 3 times. The residue was dried in vacuo for 2 h to give 2 as a pale yellow solid (1.00 g, 1.69 mmol, 48%). UV spectroscopy gave a λmax of 305 nm (ε 18,900 m 2 cm −1). The NMR data were as follows: 1H NMR δ 8.26 (2H, d, J = 4.5 Hz, o-H Ar–CH = N), 7.62–7.87 (15H, m, ArH), 7.47 (1H, s, CH = N), 6.85 (2H, d, J = 4.6 Hz, o-H Ar–O–C), 4.15 (2H, t, J = 5.4 Hz, O–CH2–C), 3.85–3.95 (2H, m, p–CH2–C), 2.26 (2H, quintet, J = 6.3 Hz, O–CH2–C) 1.91–1.92 (2H, m, p–CH2– C), 1.60 (9H, s, C–(C3)3) ppm. 13C NMR δ 25.20 ppm. ESMS found (M+) 510.2554 calculated for C33H37O2NP (M+): 510.2556. Octan-1-ol/PBS partition coefficients were determined as described at room temperature (22).

Measurement of Uptake of MitoPBN by Mitochondria—An ion-selective electrode for MitoPBN was constructed and inserted into the stirred chamber of an oxygen electrode thermostatted at 30 °C (22–24). Calibration of the electrode response by additions of MitoPBN from 10–30 μM gave a response that was a linear function of log10 [MitoPBN] with a slope of ~60 mV, as predicted by the Nernst equation.

EPR Measurements—A Bruker EMX spectrometer was used. Incubations were in an acid-washed quartz flat cell (Wilmad-Labglass, Buena, NJ) at room temperature (22–24 °C). For UV irradiation, N2-sparged samples were irradiated for 1 min using a UV-GL-58 mineral light lamp (UVP, Upland, CA) at 254 nm. For Fenton chemistry the N2-sparged samples were irradiated with 20 μM FeCl3 (40 μM NaClO3) for 2 min. Ascorbic acid was added (0.6% v/v) H2O2, 100 μM FeCl3, and 1 μM PBS. For exposure to superoxide, the same buffer was air-saturated and supplemented with 500 μM hypoxanthine, 0.1 units/ml xanthine oxidase (XO), and 500 μM MitoPBN.

Mitochondrial Preparations and Incubations—Rat liver mitochondria were prepared by homogenization and differential centrifugation (25). Oxygen electrode experiments with liver mitochondria were measured in 120 mM KCl, 10 mM HEPES, 1 mM EGTA, pH 7.2 in the 3-ml stirred and thermostatted chamber of a Clark-type oxygen electrode (Rank Brothers, Bottenham, Cambridge, UK). Rat kidney, skeletal muscle, and brown adipose tissue mitochondria were prepared as described (26). Mitochondria were suspended in 250 mM sucrose, 5 mM Tris-HCl, 2 mM EGTA, pH 7.4). For BAT mitochondria the medium was supplemented with 1% (w/v) defatted BSA, and the mitochondrial pellet was then washed twice in isolation medium without BSA. Mitochondrial pellets were suspended in isolation medium, and the protein concentration was determined by the biuret method using BSA as a standard (27).

Yeast cultures (Saccharomyces cerevisiae strain DBY746, MATa leu2-3,112 his3Δ1 trp1-289 ura3-52) were grown aerobically at 28 °C to mid-log phase in lactate-containing medium (2% lactic acid, 0.3% yeast extract, 0.05% glucose, 0.05% CaCl2·2H2O, 0.05% NaCl, 0.06% MgCl2·6H2O, 0.1% KH2PO4, 0.1% NH4Cl (all w/v), pH 5.5) (27). Cells were washed twice by centrifugation in Tris-dithiothreitol buffer (0.1 M Tris-SO4, pH 9.4, 10 mM dithiothreitol), and incubated for 20 min at 30 °C. The cells were washed twice in 1.2 M sorbitol buffer (1.2 M sorbitol, 20 mM KP, pH 7.4) and converted to spheroplasts by incubation in 1.2 M sorbitol buffer containing lyticase.
Activation of Uncoupling Proteins

**Table I**

| Compound          | Partition coefficient |
|-------------------|-----------------------|
| TPMP              | 0.380 ± 0.001         |
| PBN               | 9.4 ± 0.9             |
| MitoPBN           | 8.4 ± 0.2             |
| MitoBenzaldehyde  | 17.0 ± 0.9            |

was indistinguishable from that of freshly isolated yeast mitochondria as confirmed by the uncoupler-sensitive uptake of \(^{3}H\)TPMP (data not shown).

**Assays**—The TBARS assay was used to quantitate lipid peroxidation (14). Rat liver mitochondria (4 mg of protein) were suspended in 2 ml of buffer (100 mM KCl, 10 mM Tris-HCl, pH 7.6, 10 mM succinate, 8 μg of rotenone/ml) supplemented with ethanol carrier or test compound. After 5 min of preincubation, oxidative stress was induced by addition of 100 μM FeSO₄ and 300 μM ascorbic acid, and 40 min later the incubation was divided into two 800-μl aliquots and 400 μl of thiobarbituric acid (TBA; 0.05% w/v in 10 ml of H₂O, 10 ml of perchloric acid) was added to each aliquot. Samples were heated at 100°C for 15 min, cooled on ice, and then transferred to a glass tube, and 3 ml of water and then 3 ml of butanol were added. After vortexing, the organic layer was isolated by centrifugation, and 200-μl aliquots were analyzed in a fluorometric plate reader (λₐₐₕ 515 nm, λₐₜₜ 555 nm) and compared with a standard curve of 0–30 nmol of 1,1,3,3-tetraethoxypropane.

To assess reactivity of different molecules with the hydroxyl radical, we used ferrous iron to generate the hydroxyl radical and then measured the hydroxylation of benzoic acid (29). This was done in 30 mM NaP₂O₄, pH 7.4, 40 mM NaCl containing 690 μM sodium benzoate, 30 μM EDTA, and 200 μM FeCl₂. Compounds were incubated at 37°C for 60 min, then cooled on ice, and diluted in 3 ml of buffer, and the fluorescence was measured in a stirred 3-ml system (λₐₚₚ 305 nm, λₕₘₚ 407 nm).

To study the interaction of different compounds with superoxide, xanthine oxidase (XO; 0.01 units/ml) in 50 mM KPi, pH 7.5, 1 mM EDTA, 100 μM DTPA supplemented with 500 μM hypoxanthine was used to generate superoxide. Superoxide production was measured as the rate of reduction of 50 μM acetylated cytochrome c (Sigma) at 550 nm in a 1-ml cuvette at 30°C.

To measure the oxidation of ferrous iron, 110 μM FeCl₂ was incubated in Chelex-treated 50 mM NaCl, 5 mM Tris-HCl, pH 7, under argon. At various times 100-μl samples were removed, added to 900 μl of 1 mM Ferrozine (30) in the same buffer, and the Ferrozine-Fe(II) complex was assayed spectrophotometrically (εₐₜₜ = 27.9 × 10³ M⁻¹ cm⁻¹) (30). Fe(II) oxidation over 30 min and the absorbance of the tested compounds at 954 nm were both negligible. To confirm that oxidation of Fe(II) produced Fe(III) we used desferrioxamine, which chelates Fe(III) (desferrioxamine-Fe(III), εₐₜₜ = 2.8 × 10³ M⁻¹ cm⁻¹). This was done under argon as desferrioxamine in the presence of oxygen rapidly oxidizes Fe(II) to Fe(III). For this 1 mM desferrioxamine was stirred in an air-tight 3-ml cuvette under argon with 100 μM FeCl₂ and after an injection of TEMPO there was rapid (<1 s) oxidation of Fe(II) to Fe(III).

Aconitate activity was measured by a coupled enzyme assay linking isocitrate production by aconitate to NADPH reduction by isocitrate dehydrogenase (εₐₚₚ = NADPH = 6.22 × 10³ M⁻¹ cm⁻¹) (31, 32). The background rate of NADPH formation was determined in the presence of fluorocitrate (100 μM), a competitive inhibitor of aconitate, and was always less than 10% of the initial rate. Aliquots of frozen yeast mitochondria were thawed rapidly, washed in mannitol buffer (0.6 M mannitol, 10 mM Tris maleate, pH 6.8, 5 mM KPi, 0.5 mM EDTA), and resuspended in this buffer at 0.2–0.3 mg of protein/ml. MitoPBN or TPMP (from stocks in dimethyl sulfoxide) and substrate were added, and the mitochondria were incubated in a shaking water bath at 30°C. Samples were removed at various time points, snap-frozen on dry ice, and thawed prior to assaying. The aconitate assay was adapted for a 96-well plate format with a 10-μl sample added to 190 μl of assay buffer (50 mM Tris-HCl, pH 7.4, 0.6 mM MnCl₂, 5 mM sodium citrate, 0.2 mM NADP⁺, 0.1% w/v Triton X-100, and 0.4 units/ml isocitrate dehydrogenase) at 30°C and assayed with A₅₈₆ readings at 15-s intervals over 7 min. The resulting slopes of multiple samples (typically 6) were averaged.

The oxidation of cis-parinaric acid (cPA) was monitored fluorometrically (λₐₜₜ = 324 nm; λₚₚₚ = 413 nm) in a 3-ml fluorometer cuvette at...
37 °C with constant stirring (33). Beef heart mitochondrial membranes, prepared as described (34), were incubated in 50 mM KPi buffer, pH 8.0, and after 40 s, cis-parinaric acid (0.5 μM) was added, and its oxidation was monitored. The absorption spectrum of MitoPBN overlaps with the excitation spectrum of cPA; therefore, for comparisons all experiments were adjusted to the same maximum 100% fluorescence immediately following addition of cPA.

**Proton Leak Measurements**—Mitochondria (0.35 mg of protein/ml) from kidney or skeletal muscle were incubated in 120 mM KCl, 5 mM KP, 3 mM HEPES, and 1 mM EGTA, pH 7.2, at 37 °C, with 5 μM rotenone, 80 ng of nigericin/ml, and 1 μg of oligomycin/ml. BAT mitochondria were incubated in 50 mM KCl, 1 mM EGTA, 4 mM KP, 5 mM HEPES, pH 7.2, and 1% w/v defatted BSA at 37 °C with 5 μM rotenone, 80 ng of nigericin/ml, and 1 μg of oligomycin/ml. Respiration rate and membrane potential were measured simultaneously using electrodes sensitive to oxygen and TPMP (4). The TPMP electrode was calibrated with five sequential 0.5 μM additions of TPMP and then substrate was added, 4 mM succinate for kidney or skeletal muscle mitochondria or 10 mM α-glycerophosphate for BAT mitochondria. Membrane potential was varied by adding malonate (up to 1 mM) for kidney and skeletal muscle or KCN (up to 100 μM) for BAT mitochondria. After each run, 0.2 μM FCCP was added to release TPMP for baseline correction. When MitoPBN was used, the TPMP electrode was calibrated with five sequential additions of 9:1 TPMP:MitoPBN to final concentrations of 2.25 and 0.25 μM respectively. For simplicity, the TPMP binding correction was assumed to be 0.4/μM per mg protein (24) for mitochondria from all tissues; this will have caused small systematic errors in membrane potential in muscle mitochondria or when Mi-

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**Fig. 2. Effect of MitoPBN on the mitochondrial proton conductance catalyzed by UCP1, UCP2, and UCP3, activated by superoxide or HNE.**

*Figures A and B,* MitoPBN prevents activation of UCP2 in kidney mitochondria by exogenous superoxide. Rat kidney mitochondria were incubated with xanthine oxidase (where shown) and xanthine to generate superoxide. Membrane potential was monitored using a TPMP electrode calibrated by sequential additions of TPMP up to 2.5 μM (A) or 9.1 TPMP:MitoPBN up to 2.25 and 0.25 μM, respectively (B). Membrane potential was generated using 4 mM succinate and gradually decreased by sequential additions of malonate up to 1 mM. Xanthine oxidase (0.01 units/3.5 ml assay), GDP (0.5 mM), MitoPBN (250 nM), and PBN (250 nM) were present where indicated. Data are means ± S.E. of three independent experiments each performed in duplicate. *C and D,* MitoPBN prevents activation of UCP1 in rat BAT mitochondria by exogenous superoxide. Proton leak kinetics for BAT mitochondria were measured as described in *A and B,* except that the membrane potential was generated using 10 mM α-glycerophosphate and was gradually decreased by sequential addition of KCN (up to ~100 μM). Data are means ± range of two independent experiments each performed in duplicate. *E and F,* MitoPBN prevents activation of UCP3 in rat skeletal muscle mitochondria by exogenous superoxide. Proton leak kinetics were measured as in *A and B.* Data are means ± S.E. of three independent experiments each performed in duplicate. *G and H,* no effect of MitoPBN on HNE activation of UCP2. Proton leak kinetics were measured in rat kidney mitochondria as in *A and B* in the presence of 0.3% (w/v) defatted BSA, with or without 35 μM HNE and 250 nM MitoPBN as indicated. Data are means ± range of two independent experiments each performed in duplicate. The stimulation of leak by HNE is GDP-sensitive (5). *, respiration rate at the highest common potential in the relevant panel is significantly different (*p < 0.05,* Student’s paired t test) from control (left-hand panels) or MitoPBN (right-hand panels).
FIG. 3. Interaction of MitoPBN with reactive oxygen species and iron. A, aconitase inactivation. Mitochondria were incubated in the presence or absence of paraquat (10 mM); samples were removed at various times, and aconitase activity was measured and expressed as a percentage of the activity at $t = 0$. Data are for yeast mitochondria incubated with $10 \mu M$ MitoPBN or TPMP respiring on succinate (10 mM) and are the means ± range of two independent experiments. B, effect of exogenous superoxide on aconitase activity. Rat liver mitochondria (1 mg of protein/ml) were incubated in KCl buffer plus DTPA with 500 $\mu M$ hypoxanthine, 40 units/ml catalase, 0.01 units/ml xanthine oxidase, 10 $\mu M$ MitoPBN. Mitochondria (250 $\mu g$ of protein) were then pelleted and assayed for aconitase activity. Data are means ± range of duplicates from a typical experiment that was repeated 3 times with identical results. C, reactivity of MitoPBN with hydroxyl radical. Ferrous iron was used to generate a flux of hydroxyl radicals, which was assessed as the increase in fluorescence on hydroxylation of benzoic acid. The decreases in fluorescence in the presence of test compounds were expressed as a percentage of control incubations. Data are means ± S.D. of three independent experiments. D, anaerobic oxidation of ferrous iron. FeCl$_2$ (110 $\mu M$) in 50 mM NaCl, 5 mM Tris-HCl, pH 7, was incubated anaerobically with 500 $\mu M$ MitoPBN, 500 $\mu M$ TPMP, 10 $\mu M$ PBN, or 100 $\mu M$ TEMPO. Samples were taken at various times, and [Fe(II)] was assayed using FerroZine. Data are means ± S.D. of duplicate determinations. The Fe(II) lost on reaction with TEMPO was recovered as Fe(III), assayed using desferrioxamine as described under “Experimental Procedures.” None of these compounds reacted with 110 $\mu M$ FeCl$_3$ under anaerobic conditions (not shown). E, interaction of MitoPBN with ferrous iron in the presence of oxygen. An aerobic incubation was started by adding FeCl$_2$ (110 $\mu M$) from an anaerobic stock to 50 mM NaCl, 5 mM Tris-HCl, pH 7, and the loss of [Fe(III)] was measured over time as described above. This was repeated in the presence of 500 $\mu M$ MitoPBN or the indicated concentrations of PBN. Data are means ± S.D. of three independent experiments. *, $p < 0.05$; **, $p < 0.001$ for changes relative to controls by Student’s t test. F, oxidative damage to the respiratory chain. Rat liver mitochondria (1 mg of protein/ml) were incubated with 20 mM paraquat or 5 mM tert-butylhydroperoxide (tBHP) for 10 min at 30°C in sucrose-based isolation medium.
toPBN was present. Exogenous superoxide was generated using xanthine (50 μM) and xanthine oxidase (0.01 units/5.5 ml assay) (4). Xanthine and xanthine oxidase were added before the TPMP (or TPMP:MitoPBN) calibration and incubated with mitochondria for 10–15 min before addition of substrate. To assess the statistical significance of the shifts in leak curves caused by superoxide, we generally compared respiration rates at the highest common membrane potential for pairs of curves from 3 independent experiments using Student’s t test for paired data.

RESULTS AND DISCUSSION

Synthesis of MitoPBN—Acyclic nitrones such as PBN are usually formed by reductive condensation of a benzaldehyde with a nitroalkane (16, 20). Nitrene formation is a slow equilibrium process that can be shifted in favor of the nitrene by dehydrating agents. The phosphonium unit could be attached to the basic PBN core at a number of positions via carbon or heteroatom, and the length of the alkyl chain between PBN and the phosphonium unit is variable. Anticipating adverse sensitivity of the nitrorene to chemical manipulation, the phosphonium functionality was introduced first using a phenoxyalkylation methodology (35) to link (4-iodobutyl)triphenylphosphonium iodide to para-hydroxybenzaldehyde before incorporating the nitrogen function in the final step (Scheme 1). Reaction of (4-iodobutyl)triphenylphosphonium iodide with the anion derived from para-hydroxybenzaldehyde gave Mito-Benaldehyde (1) in 84% yield (Scheme 1). Reductive condensation of 1 with 2-methyl-2-nitropropane under conditions optimized using para-methoxybenzaldehyde and based on reported methodology (20) gave low yields of MitoPBN (2). The reaction was modified and carried out in dry absolute alcohol in the presence of molecular sieves (16) and over 9 days gave pure 2 in 48% yield, as indicated by 1H and 31P NMR analysis.

Uptake of MitoPBN by Mitochondria—To determine whether MitoPBN was accumulated by energized mitochondria, an insensitive electrode for MitoPBN was used. In Fig. 1 the electrode response below 5 μM was calibrated by sequential MitoPBN additions, and then a membrane potential was generated by addition of succinate, leading to accumulation of MitoPBN by mitochondria. Dissipation of the membrane potential with the uncoupler FCCP led to the immediate release of MitoPBN from the matrix (Fig. 1). The external concentration of MitoPBN following uptake was 0.91 ± 0.08 μM indicating that mitochondria had accumulated 2.0 ± 0.2 nmol of MitoPBN/mg of protein (n = 3). The mitochondrial volume under these conditions (0.5–0.9 μl/mg) (36–39) gives an intramitochondrial MitoPBN concentration of 2.2–4 mM. The Nernst equation implies that this accumulation ratio of 2,400–4,400-fold corresponds to a membrane potential greater than the expected value of −180 mV (24). This overestimation is due to MitoPBN binding reversibly to the matrix surface of the inner membrane (24, 39, 40); correcting for the expected potential suggests that 65–75% of accumulated MitoPBN is bound, compared with ~60% for the TPMP (36) and is consistent with their relative hydrophobicities (Table 1).

To see if MitoPBN disrupted mitochondrial function, we compared its effects on respiration of rat liver mitochondria with those of TPMP, PBN, and with Mitobenzaldehyde (1), a possible breakdown product of MitoPBN (41). None of these compounds affected resting, phosphorylating, or uncoupling respiration at 10 μM or lower, but there were minor inhibitory and uncoupling effects of the triphenylphosphonium containing compounds at 25 μM and above (data not shown). Therefore, MitoPBN concentrations of 25 μM to 10 μM were used for most of the experiments reported here, and controls with TPMP were done to check that there were no nonspecific effects on mitochondria.

MitoPBN Blocks Activation of UCP1, UCP2, and UCP3 by Superoxide but Not by HNE—Fig. 2A shows that kidney mitochondria exposed to exogenous superoxide from xanthine and xanthine oxidase showed an increase in proton conductance that was fully prevented by the specific UCP inhibitor, GDP, as reported previously (4). The superoxide activation of UCP2 was completely abolishes by 250 nM MitoPBN (Fig. 2B). However, the same concentration of PBN did not affect the superoxide-induced proton conductance (Fig. 2A). 25 nM MitoPBN also blocked the superoxide-stimulated leak, and even 2.5 nM MitoPBN attenuated the effect, whereas PBN concentrations of at least 10 μM were required to block superoxide activation (data not shown). This more than 400-fold increased potency of MitoPBN over PBN can be explained by the accumulation of MitoPBN within the mitochondrial matrix, as demonstrated in Fig. 1. N-tert-Butylhydroxylyamine, a hydrolysis product of PBN that accumulates in PBN stock solutions, accounts for some of the protective effects of PBN in cell culture (41); however, 100 μM N-tert-butylhydroxylyamine did not affect superoxide-induced proton conductance (data not shown). Therefore, this breakdown product does not contribute to the effects of MitoPBN on superoxide-induced leak.

Kidney mitochondria contain only UCP2 (4, 42), so the experiments in Fig. 2, A and B, show that MitoPBN prevents superoxide activation of UCP2. To see if MitoPBN also blocked

Supplemented with rotenone (6 μg/ml), succinate (5 mM), and 5 μM MitoPBN or TPMP. The mitochondria were then isolated by centrifugation, resuspended at 1 mg of protein/ml in 3 ml of 120 mM KCl, 10 mM HEPES, 1 mM EGTA, pH 7.2, containing 100 μM DTPA, 5 mM succinate, and 1 μM FCCP, and the rate of uncoupled respiration was measured in an oxygen electrode chamber at 30 °C. Data are percentages of untreated controls and are means ± range of two independent experiments, each done in duplicate. The respiration rate of controls was 79 ± 18 nmol of O2/min/mg protein.
the superoxide stimulation of proton leak by other UCPs, we investigated mitochondria from BAT (Fig. 2, C and D) and skeletal muscle (Fig. 2, E and F). Superoxide activation of proton conductance in BAT mitochondria operates primarily through UCP1 (4). As reported previously (4), the basal activity of UCP1 in BAT mitochondria was inhibited by GDP because UCP1, unlike UCP2 and UCP3, is activated in the absence of superoxide by endogenous factors including fatty acids. As before (4), this basal proton conductance of UCP1 was further stimulated by superoxide in a GDP-sensitive manner (Fig. 2C).

MitoPBN prevented this activation (Fig. 2D), so it prevents superoxide activation of UCP1. Superoxide activation of proton conductance in skeletal muscle mitochondria operates through UCP3, because the effect is absent in mitochondria from UCP3−/− mice (4). Superoxide activated proton conductance in skeletal muscle mitochondria (Fig. 2E) as reported previously (4). MitoPBN prevented this activation (Fig. 2F), so it also blocks superoxide activation of UCP3. Thus, the abolition of superoxide activation of UCPs by MitoPBN is general to all three mammalian UCPs.

Lipid peroxidation products such as HNE activate UCPs by a GDP-sensitive mechanism (5). In contrast to its inhibitory effect on superoxide-activated proton conductance, MitoPBN had no effect on the activation of UCP2 by HNE in kidney mitochondria (Fig. 2, G and H).

The mitochondria-targeted spin trap MitoPBN blocks the superoxide-induced increase in UCP proton conductance with more than 400-fold greater potency than the untargeted PBN because it is accumulated within the mitochondria. This finding indicates that MitoPBN blocks the UCP activation pathway within the matrix. This conclusion is consistent with our earlier findings that the mitochondria-targeted antioxidants MitoQ and MitoVit E act within mitochondria to prevent superoxide activation of UCPs (12). In contrast, MitoPBN did not affect the stimulation of proton conductance by HNE. This difference suggests two scenarios: either superoxide activates UCPs by generating lipid peroxidation products such as HNE, and MitoPBN prevents superoxide activation by blocking the production (but not the activity) of such lipid peroxidation products; or the stimulation of UCP proton conductance by superoxide and HNE occur by distinct processes. To distinguish between these possibilities, we next investigated how MitoPBN interacts with the ROS generated within mitochondria exposed to superoxide.

**Interactions of MitoPBN with Reactive Oxygen Species**

When superoxide was generated from xanthine and xanthine oxidase in the absence of mitochondria, MitoPBN concentrations up to 500 μM did not decrease the superoxide dismutase-sensitive reduction of cytochrome c (data not shown); therefore, MitoPBN does not react significantly with superoxide. To see if MitoPBN affected the reactivity of superoxide within the mitochondrial matrix, we measured the rate of inactivation of the matrix enzyme aconitase, which is particularly sensitive to damage by superoxide (32). Neither the spontaneous inactivation of aconitase by endogenous superoxide nor the high rate of inactivation induced by the redox cycler paraquat were prevented in yeast mitochondria by MitoPBN (Fig. 3A).

During the proton leak measurements shown in Fig. 2, deenergized mitochondria were exposed to exogenous superoxide which moves into the matrix, presumably by passage of its conjugate acid (pK 4.8) (43) through the phospholipid bilayer xanthine plus 0.01 units/ml XO at 37°C in the presence or absence of 200 μM desferrioxamine (desfer), and cPA was added after 1.5 min, and its fluorescence decay was measured. A typical experiment is shown; two independent repeats gave similar results, as did replacing desferrioxamine with an alternative iron chelator DTPA (2 mM).
(12). MitoPBN did not affect the inactivation of aconitase in de-energized rat liver mitochondria exposed to exogenous superoxide (Fig. 3B); therefore, MitoPBN does not inhibit superoxide movement through the mitochondrial membrane. Thus the effects of MitoPBN on the superoxide-induced leak are not due to it reacting with superoxide, preventing its uptake into mitochondria or affecting its reactivity within the matrix.

Superoxide dismutates to hydrogen peroxide, which generates the very reactive hydroxyl radical in the presence of ferrous ions. To assess the reactivity of MitoPBN with the hydroxyl radical, we measured its ability in vitro to prevent hydroxylation of benzoic acid by hydroxyl radicals generated by the Fenton reaction (29) (Fig. 3C). MitoPBN, PBN, and TPMP trapped the hydroxyl radical with IC_{50} values of ~77, ~143, and ~419 μM, respectively, whereas an equimolar mixture of PBN and TPMP gave an IC_{50} of ~100 μM (Fig. 3C).

Therefore, the reactivity of MitoPBN with the hydroxyl radical is marginally greater than that of PBN, probably due to the bulky triphenylphosphonium group. The rate constant for the reaction of PBN with the hydroxyl radical is 6.1—8.5 × 10^{10} M^{-1}s^{-1} (44, 45), suggesting that MitoPBN reacts with the hydroxyl radical at close to the diffusion limit, in common with most organic compounds (46). The IC_{50} for TPMP was only ~5.4-fold greater than that of MitoPBN, but TPMP concentrations 200-fold greater than those of MitoPBN had no effect on the activation of UCPs by superoxide. Therefore, although MitoPBN does react rapidly with the hydroxyl radical, this is not how it blocks the activation of proton leak by superoxide.

Mitochondria exposed to superoxide release ferrous iron from aconitase and other FeS proteins. This ferrous iron can then catalyze the initiation of lipid peroxidation (47, 48). Therefore, the possibility that MitoPBN could prevent UCP activation by intercepting ferrous iron was addressed. There was no reaction between MitoPBN and ferrous iron in vitro under anaerobic conditions (Fig. 3D). Free radicals react with nitrones such as MitoPBN to generate short lived nitroxides, and the stable nitroxide TEMPO rapidly oxidized ferrous to ferric iron (Fig. 3D). However, when ferrous iron was incubated aerobically to generate ROS and transient nitroxides, MitoPBN did not stimulate its oxidation, even at 500 μM (Fig. 3E). Only when PBN was added at very high concentrations (100 mM) did it affect iron oxidation. Hence MitoPBN does not prevent UCP activation by interacting with the ferrous iron released on exposure of mitochondria to superoxide.

To see if MitoPBN protected the respiratory chain from oxidative damage, we exposed rat liver mitochondria to superoxide by using the redox cycler Paraquat, or we oxidized the glutathione pool with the glutathione peroxidase substrate tert-butylhydroperoxide. Both treatments substantially decreased the rate of uncoupled respiration due to generalized oxidative damage to the respiratory chain, but MitoPBN gave no protection against either form of oxidative damage (Fig. 3F). In summary, MitoPBN does not prevent superoxide peroxidation in mitochondria by reacting with superoxide, ferrous iron, or the hydroxyl radical, or through general antioxidant protection.

**Trapping of Carbon-centered Radicals by MitoPBN—PBN reacts rapidly with carbon-centered radicals, so we next determined whether MitoPBN also underwent this reaction. UV photolysis of H_2O_2 in ethanol generated hydroxyl radicals that reacted rapidly with ethanol to yield the carbon-centered α-hydroxyethyl radical (17). PBN gave the well characterized α-hydroxyethyl radical adduct shown in Fig. 4 (AN = 15.37 ± 0.06; A_{477} = 3.62 ± 0.04; A_{270}/A_{477} = 4.24) (49). The α-hydroxyethyl radical also reacted with MitoPBN to give a radical adduct with hyperfine splitting constants similar to those of PBN (AN = 15.43 ± 0.08; A_{477} = 3.47 ± 0.01; A_{270}/A_{477} = 4.4) (Fig. 4), and when a mixture of MitoPBN and PBN was exposed to α-hydroxyethyl radicals, the spectra were overlapping and additive (data not shown). However, whereas the α-hydroxyethyl radical adduct of PBN was long lived with negligible loss over 80 min, that of MitoPBN decayed more rapidly, with no signal detectable from 1 mM MitoPBN ~40 min after UV irradiation (data not shown). The faster decay was not due to an intermolecular interaction between the cation and the α-hydroxyethyl radical adduct of PBN, as UV irradiation of an equimolar mixture of TPMP and PBN generated a long lived radical adduct of PBN (data not shown). However, radical adducts of para-methoxy-PBN decay more rapidly than those of PBN due to the electron-donating methoxy group (50). Thus the faster decay of MitoPBN radical adducts is probably a consequence of the electron-donating ether linkage between the PBN moiety and the triphenylphosphonium. No oxygen-centered radical adducts were detected when 500 μM MitoPBN was exposed to hydroxyl radicals generated by the Fenton reaction or to superoxide generated by xanthine oxidase/hypoxanthine (data not shown), consistent with the short lifetime of the adducts formed between PBN and oxygen-centered radicals and the low rate of reaction between PBN and superoxide (16). Therefore, the rapid reaction of MitoPBN with carbon-centered radicals may be how MitoPBN blocks the activation of UCPs by superoxide. As carbon-centered radicals occur during the initiation of lipid peroxidation, we next investigated the effects of MitoPBN on lipid peroxidation.

**Effects of MitoPBN on Lipid Peroxidation—**Lipid peroxidation is initiated by abstraction of a hydrogen atom to generate a carbon-centered radical on phospholipid fatty acyl chains. These carbon-centered radicals react with oxygen to form lipid hydroperoxides, which drive a self-propagating chain reaction. Once initiated, the lipid peroxidation chain reaction can be prevented by chain-breaking antioxidants such as vitamin E or ubiquinol. PBN, however, is a poor chain-breaking antioxidant (18). MitoPBN was also ineffective at preventing lipid peroxidation in liver mitochondria exposed to ferrous iron/ascorbate (Fig. 5A), in contrast to MitoVit E and MitoQ (14, 15). This observation suggests that MitoPBN cannot block lipid peroxidation once it is initiated by excess pro-oxidant. Although PBN is ineffective as a chain-breaking antioxidant, it can prevent initiation of lipid peroxidation by reaction with carbon-centered radicals (18). As MitoPBN reacts rapidly with carbon-centered radicals (Fig. 4), we next set out to test whether it too could block the initiation of lipid peroxidation by reacting with carbon-centered radicals.

![Scheme 2](image)
Exposing mitochondrial membranes to superoxide also decreased cPA fluorescence, and this reaction too was blocked by MitoPBN (Fig. 5C). Superoxide-induced lipid peroxidation in mitochondrial membranes was prevented by the iron chelator desferrioxamine (Fig. 5D), suggesting that superoxide alone was unable to initiate lipid peroxidation. In these experiments the iron probably became associated with the membranes during preparation and storage, as well as being released from FeS centers within respiratory complexes on exposure to superoxide. The superoxide anion is insufficiently reactive to initiate lipid peroxidation by abstraction of a hydrogen atom from a fatty acid (51). Its conjugate acid, the hydroperoxyl radical, can initiate lipid peroxidation; however, the rate of hydrogen atom abstraction from isolated fatty acids by this radical \( k \approx 10^6 - 10^7 \text{ M}^{-1}\text{s}^{-1} \) (17, 45). In contrast, MitoPBN will not stop lipid peroxidation once initiated by other sources. This is because the rate of reaction of PBN with the peroxyl radicals essential for propagating lipid peroxidation is low \( k \approx 40 - 200 \text{ M}^{-1}\text{s}^{-1} \) (52), and the radical adducts thus formed are very unstable (18).

**FIG. 6.** A model for the activation of UCPs by superoxide through initiation of lipid peroxidation. Superoxide \( \text{O}_2^- \) generated outside mitochondria by XO, or within mitochondria by the respiratory chain, inactivates iron-sulfur center-containing enzymes such as aconitase, expelling ferrous iron. The superoxide is also dismutated (mostly by the matrix Mn-superoxide dismutase) to hydrogen peroxide \( \text{H}_2\text{O}_2 \), which reacts with ferrous iron by the Fenton reaction to generate the hydroxyl radical \( \cdot\text{OH}\). The hydroxyl radical extracts a hydrogen atom \( \text{H}^- \) from a bis-allylic position on an unsaturated fatty acyl chain of a phospholipid, leaving a carbon-centered fatty acyl radical that can be quenched by spin traps such as MitoPBN. The carbon-centered radical reacts with oxygen to initiate formation of a peroxy radical, which then propagates a chain reaction of lipid peroxidation leading to a complex mixture of lipid peroxidation breakdown products. Two of these, 4-hydroxynonenal (HNE) and 4-hydroxyhexenal, are shown. HNE and other lipid peroxidation breakdown products activate UCPs, thereby increasing the proton conductance of the mitochondrial inner membrane. This activation of UCPs decreases the protonmotive force, and we have proposed elsewhere (7) that is part of a regulatory mechanism to decrease superoxide production by the respiratory chain when ROS production is too high.
UCP Activation by Superoxide Exposure Leading to Lipid Peroxidation Products—MitoPBN blocks superoxide activation of UCPs, but this is not due to its reaction with superoxide, iron, or the hydroxyl radical. As MitoPBN reacts rapidly with carbon-centered radicals, one possibility is that superoxide acts within mitochondria to generate carbon-centered radicals on phospholipid acyl chains. How this might occur is outlined in Fig. 6. Mitochondria exposed to endogenous or exogenous superoxide undergo inactivation of iron-sulfur center proteins, such as aconitase or respiratory chain complexes, expelling ferrous iron (48). This ferrous iron reacts with hydrogen peroxide, produced by dismutation of superoxide catalyzed mostly by mitochondrial Mn-superoxide dismutase, to generate hydroxyl radicals by Fenton chemistry. These hydroxyl radicals attack the fatty acyl chains of mitochondrial phospholipids to initiate formation of carbon-centered radicals. The carbon-centered radicals then react with oxygen to form peroxy radicals, which in turn propagate a cascade of lipid peroxidation. Depending on the fatty acyl chain that is attacked, and on the particular breakdown pathway that ensues, lipid peroxidation leads to the formation of large amounts of reactive small lipid fragments such as HNE (from n-6 fatty acyl groups such as 20:4(n-6), arachidonyl), hydroxyhexenal (from n-3 fatty acyl groups such as 22:6(n-3), docosahexaenoyl), and malondialdehyde (53), most or all of which can activate the proton conductance of UCPs.

This scenario explains how MitoPBN can act at the beginning of this cascade to intercept carbon-centered radicals and prevent the initiation of lipid peroxidation. It also explains why MitoPBN did not prevent the activation of UCPs by HNE, which occurs downstream. Most importantly, this model indicates how the apparently unrelated activation of UCPs by superoxide and HNE can occur by a common pathway. MitoQ and MitoVit E also block superoxide-induced leak, but as they can act as chain-breaking antioxidants, and also react with superoxide, their effect on superoxide activation of UCPs is far less informative than that of MitoPBN.

As well as being consistent with the data presented here, the model in Fig. 6 has testable predictions. One prediction is that the release of iron within mitochondria is necessary for superoxide to initiate lipid peroxidation and UCP activation; another is that the generation of carbon-centered radicals in the phospholipid bilayer would lead to UCP activation and that this should be prevented by MitoPBN.

Requirement for Mitochondrial Iron for Superoxide Activation of UCPs—The initiation of lipid peroxidation when mitochondria are exposed to superoxide probably arises from the release of ferrous iron from FeS centers such as aconitate (48), as the direct initiation of lipid peroxidation by superoxide is slow, even when it is protonated (51). Therefore, we investigated whether addition of iron chelators affected the activation of UCPs by superoxide. Most of the iron chelators we investigated could not be used during measurements of proton conductance of isolated mitochondria due to membrane impermeance, excessive uncoupling, or limited solubility. Furthermore, many chelators have complicated interactions with ROS (54). However, we found that bipyridyl was usable up to 5 mM, although it did cause considerable nonspecific uncoupling, limiting the concentrations that could be tested. Bipyridyl attenuated the superoxide activation of proton conductance through UCP2 in kidney mitochondria (data not shown), consistent with superoxide acting to increase leak via changes in intramitochondrial iron.

Activation of UCPs by Carbon-centered Radicals—If superoxide activates UCPs by generating carbon-centered radicals within the phospholipid bilayer, then generating such radicals directly should activate UCPs. To test this we added the carbon-centered radical generator AAPH to kidney mitochondria. 2 mM AAPH strongly increased the proton conductance, and this activation was fully prevented by addition of GDP, indicating that AAPH activated UCP2 and did not uncouple by causing nonspecific damage to the mitochondria (Fig. 7A). Carboxyatractylate, a specific inhibitor of the adenine nucleotide translocase, also prevented activation by AAPH (Fig. 7A). These results faithfully echo the effects on UCPs and the adenine nucleotide translocase seen previously with HNE (5). MitoPBN completely blocked this activation of UCP2 by AAPH (Fig. 7B), suggesting that MitoPBN was able to prevent initiation of lipid peroxidation and hence the activation of UCP2 by AAPH. Together these data are consistent with the general model (Fig. 6) that superoxide activates UCPs through the generation of carbon-centered radicals within mitochondria and that MitoPBN blocks this by preventing the initiation of lipid peroxidation through reaction with carbon-centered radicals.

Conclusions—In the work reported here, MitoPBN was synthesized by generating a phenoxide from p-hydroxybenzaldehyde, which was then coupled to the butyltriphenylphosphonium
moiety of (4-iodobutyl)triphenylphosphonium through an ether linkage by nucleophilic displacement of iodide (Scheme 1). The benzaldehyde (1) was then converted to a nitroxide to give MitobPN (2). Introducing the active moiety after conjugation to the lipophilic cation contrasts with the conventional approach of creating the triphenylphosphonium cation in the last step in the synthesis by reaction of triphenylphosphine with a halogenated precursor (14, 15, 40). The success of this procedure expands the number of possible synthetic routes to mitochondria-targeted molecules.

MitobPN has proven to be a powerful tool to probe pathways of ROS-induced changes in isolated mitochondria. It blocks the activation of proton conductance by superoxide from the matrix side of the mitochondrial inner membrane, but it does not affect the stimulation of conductance by the lipid peroxidation product HNE. The prevention of UCP activation by superoxide is not due to the reaction of MitobPN with superoxide, iron, or the hydroxyl radical, or by MitobPN acting as a chain-breaking antioxidant. However, MitobPN does react strongly with carbon-centered radicals. This reactivity prevents the initiation of lipid peroxidation by superoxide, and the activation of UCPs by a carbon-centered radical generator. Therefore, superoxide probably stimulates UCPs from within mitochondria by attacking and inactivating iron-sulfur center enzymes, leading to the release of ferrous iron. In the presence of hydrogen peroxide, this leads to the formation of hydroxyl radicals, which generate carbon-centered radicals on phospholipids. These carbon-centered radicals undergo further reactions and degrade to form lipid peroxidation breakdown products such as HNE, which go on to activate UCPs.

This model shows how the activation of UCPs by superoxide and through lipid peroxidation breakdown products such as HNE lie on the same pathway (Fig. 6). However, the way in which such products activate UCPs is still unclear. The physiological role of activation of mild uncoupling by UCP2 and UCP3 through the superoxide-initiated lipid peroxidation pathway may be to lower the protonmotive force and decrease endogenous superoxide production in the mitochondrial matrix. This mechanism provides a simple negative feedback loop, with HNE and other lipid peroxidation products as the mediators, to ensure that superoxide production in the mitochondrial matrix is minimized, at the expense of the efficiency of energy conservation (7).

PBN has a number of protective pharmacological properties in vivo, although the mechanism of these effects is often unclear, they are not always associated with general antioxidant efficacy (reviewed in Refs. 55 and 56). Our finding of a dramatic biological effect of a PBN derivative without significant general antioxidant efficacy suggests that some of the pharmacological effects of PBN may occur by preventing the initiation of lipid peroxidation by carbon-centered radicals and by interfering with signaling cascades that rely on such generation. The selectivity of MitobPN for mitochondrial carbon-centered radicals suggests that it may be a promising candidate to alter mitochondrial oxidative damage and UCP function in vivo.

In summary, we have synthesized a novel mitochondria-targeted spin trap, MitobPN, and used it to show that superoxide activates UCPs through the formation of carbon-centered radicals and to infer that the activation of UCPs by superoxide and lipid peroxidation breakdown products such as HNE occurs on the same pathway. These findings further support the hypothesis that a major function of UCPs is to lower the mitochondrial membrane potential and thereby decrease ROS production in response to increased mitochondrial ROS or oxidative damage.
49. Li, A. S. W., Cummings, K. B., Roethling, H. P., Buettner, G. R., and Chignell, C. F. (1988) *J. Magn. Reson.* **79**, 140–142
50. Janzen, E. G., Hinton, R. D., and Kotake, Y. (1992) *Tetrahedron Lett.* **33**, 1257–1260
51. Aikens, J., and Dix, T. A. (1991) *J. Biol. Chem.* **266**, 15091–15098
52. Ohto, N., Niki, E., and Kamiya, Y. (1977) *J. Chem. Soc. Perkin Trans. II* **13**, 1770–1774
53. Esterbauer, H., Schaur, R. J., and Zollner, H. (1991) *Free Radic. Biol. Med.* **11**, 81–128
54. Cooper, C. E., Green, E. S., Rice-Evans, C. A., Davies, M. J., and Wrigglesworth, J. M. (1994) *Free Radic. Res.* **20**, 219–227
55. Floyd, R. A. (1999) *Proc. Soc. Exp. Biol. Med.* **222**, 236–245
56. Kotake, Y. (1999) *Antioxid. Redox Signal.* **1**, 481–499
Superoxide Activates Uncoupling Proteins by Generating Carbon-centered Radicals and Initiating Lipid Peroxidation: STUDIES USING A MITOCHONDRIA-TARGETED SPIN TRAP DERIVED FROM α-PHENYL-N-tert-BUTYLNITRONE

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