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INHIBITION OF MITOCHONDRIAL ELECTRON TRANSPORT BY NOR-DIHYDROGUAIARETIC ACID (NDGA)*

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Abstract—The effects of various anti-oxidants on the activities of the mitochondrial NADH-oxidase and succinoxidase systems were determined. It was concluded that nor-dihydroguaiaretic acid (2,3-dimethyl-1,4-di [3,4-dihydroxyphenyl] butane) (NDGA) was a potent inhibitor of both mitochondrial electron transport systems. NDGA did not inhibit mitochondrial cytochrome oxidase activity or the coenzyme Q–cytochrome *c* reductase system. The probable sites of inhibition of mitochondrial electron transport are complexes I and II (NADH–coenzyme Q reductase and succinate–coenzyme Q reductase systems).

NOR-DIHYDROGUAIARETIC acid (NDGA) has been implicated in cancer therapy.[1, 2] Burk and Woods[2] reported that NDGA inhibited aerobic and anaerobic glycolysis and respiration of Ehrlich ascites, K-2 ascites and leukemia L-1210 cells *in vitro*. These authors concluded that NDGA maintained the cellular pyridine nucleotides in such a reduced state that anaerobic glycolysis was inhibited.[2] The mechanism of these inhibitions remains obscure. The present paper describes some preliminary observations concerning the effect of NDGA on mitochondrial electron transport.

METHODS

Heavy beef heart mitochondria (HBHM) were isolated and prepared as previously described.[3] The activity of the succinoxidase and NADH–oxidase systems was determined manometrically in the absence and presence of the potential inhibitors.[4, 5] The various anti-oxidants, electron transport carriers and inhibitors were added in ethanol or water depending on their solubility. The ethanol concentration was kept constant in all of the assay flasks (0·1 ml of ethanol in 3 ml of reaction mixture). The activity of the coenzyme Q–cytochrome *c* reductase system[6] was determined as described in HBHM.[3]

Protein was determined by the biuret method.[7]

MATERIALS

The NDGA and coenzyme Q_{10} were kindly supplied by Dr. Charles Smart of the

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University of Utah Medical School, and Dr. Karl Folkers of the Institute for Biomedical Research, University of Texas respectively. Thiodiproprionic acid, N,N,N^1,N^1-tetramethyl-*p*-phenylenediamine (TMPD), ascorbic acid, propyl gallate and 6-*t*-butyl-*m*-cresol were purchased from Eastman Organic Chemicals. Cytochrome-*c* type III, reduced-nicotine adenine dinucleotide (NADH), succinate, α-tocopherol, antimycin and 2-*N*-heptyl-4-hydroxyquinoline-*N*-oxide (HOQNO) were purchased from Sigma. Rotenone and butylated hydroxy toluene were purchased from Aldrich Chemical Co. and Calbiochem respectively.

RESULTS AND DISCUSSION

The effects of various established anti-oxidants on HBHM NADH-oxidase and succinoxidase systems are reported in Tables 1 and 2 respectively. The data presented in Table 1 demonstrate that 100 mμmoles of butylated hydroxytoluene, 6-*t*-butyl-*m*-cresol, α-tocopherol and NDGA depressed the activity of the NADH-oxidase system

TABLE 1. EFFECT OF VARIOUS ANTI-OXIDANTS ON THE BEEF HEART MITOCHONDRIAL NADH-OXIDASE SYSTEM

Compound added (100 mμmoles/flask)	Enzyme specific activity (μatoms oxygen consumed/min/mg protein)*			Per cent†
	I	II	III	
None	0·710	0·888	0·786	100
Butylated hydroxy toluene	0·203	0·380	0·355	30–45
6-*t*-Butyl-*m*-cresol	0·321	0·363	0·406	40–50
Propyl gallate	0·558	0·687	0·676	75–85
Thiodiproprionic acid	0·761	0·786	0·769	90–110
Coenzyme Q10	1·082	1·293	1·209	145–155
α-Tocopherol	0·338	0·423	0·406	45–55
NDGA	0·118	0·101	0·144	10–20
Ascorbic acid		0·651	0·769	75–100

* Each flask contained 0·53 mg of protein.
† Per cent of uninhibited controls.

TABLE 2. EFFECT OF VARIOUS ANTI-OXIDANTS ON THE BEEF HEART SUCCINOXIDASE SYSTEM

Compound added (100 mμmoles/flask)	Succinoxidase specific activity (μatoms oxygen consumed/min/mg protein)*			Per cent†
	I	II	III	
None	0·712	0·356	0·353	100
Butylated hydroxy toluene	0·768	0·323	0·334	90–105
6-*t*-Butyl-*m*-cresol	0·703	0·330	0·335	95–100
Propyl gallate	0·339	0·151	0·110	40–55
Thiodipropionic acid	0·728	0·336	0·369	95–105
Coenzyme Q_{10}	0·762	0·348	0·344	95–110
α-Tocopherol	0·739	0·304	0·319	85–105
NDGA	0·064	0·015	0·009	2–10
Ascorbic acid	0·731	0·314	0·347	90–105

* Each flask contained from 0·53 to 1·0 mg of protein.
† Per cent of uninhibited controls.

to 35, 45, 50 and 15 per cent of the uninhibited controls respectively. The remaining anti-oxidants possessed little or no inhibitory properties on HBHM NADH-oxidase system *in vitro*, at the same concentrations. Similarly, the data presented in Table 2 show that propyl gallate and NDGA depressed the activity of the HBHM succinoxidase system to 45 and 5 per cent of the uninhibited controls respectively. The remaining anti-oxidants caused little or no inhibition at the concentrations employed.

NDGA is the most potent inhibitor of all of the anti-oxidants tested and is the only compound tested that inhibits both the HBHM NADH-oxidase and succinoxidase systems at the concentration employed. These findings are interpreted as meaning that NDGA specifically inhibited mitochondrial electron transport, an inhibition apparently not due to a nonspecific anti-oxidant effect. The data presented in Fig. 1

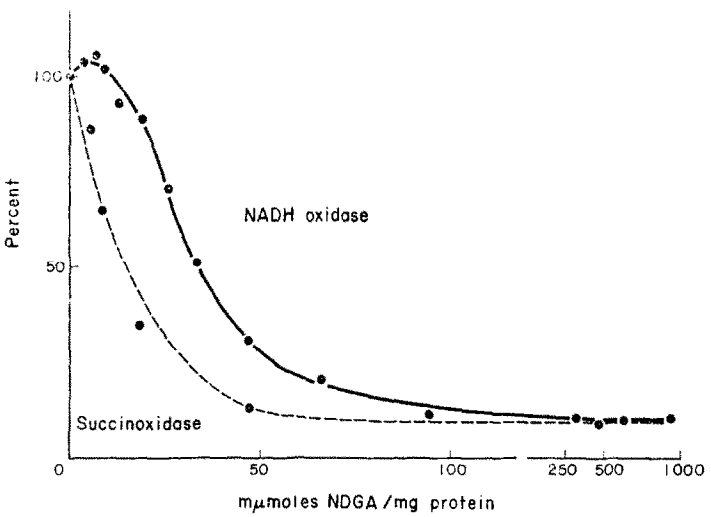

FIG. 1. Titration curve. Plot of NDGA concentration versus the activity of the NADH-oxidase and succinoxidase systems (per cent activity of uninhibited controls).

indicate that the titration curve of inhibition for NDGA is sigmoid in nature for the NADH-oxidase system and hyperbolic for the succinoxidase system. The effect of NDGA on cytochrome oxidase (Ferrocytochrome *c*:oxygen oxidoreductase; EC 1.9.3.1) activity was determined by assessing the ability of *N,N,N*^{1},*N*^{1}-tetramethyl-*p*-phenylenediamine (TMPD) to bypass the inhibition of the NADH-oxidase system caused by NDGA. This approach is based on the principle that exogenous NADH is nonenzymatically oxidized by TMPD, which in turn feeds electrons back to the respiratory chain after cytochrome b,[8] permitting cytochrome *c* to participate in electron transport through the cytochrome oxidase reaction.

The inability of TMPD to bypass the inhibition of the NADH-oxidase system by NDGA will reflect the ability of NDGA to inhibit electron transport at the cytochrome oxidase step. The data presented in Table 3 show that the inhibition of electron transport by rotenone, antimycin, 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide and NDGA was bypassed by TMPD. The inhibition caused by cyanide was not bypassed by TMPD. These findings demonstrate that the site of inhibition of electron transport by

NDGA is not at the cytochrome-oxidase step. The ability of NDGA to inhibit at complex III (Coenzyme Q–cytochrome *c* reductase system) was measured and the data presented in Table 4. These preliminary findings on HBHM indicate that NDGA does not inhibit the coenzyme Q–cytochrome *c* reductase system at an NDGA/protein concentration ratio that inhibits the NADH-oxidase system.

TABLE 3. EFFECT OF *N,N,N*^{1},*N*^{1}-TETRAMETHYL-*p*-PHENYLENEDIAMINE (TMPD) ON THE INHIBITION OF MITOCHONDRIAL ELECTRON TRANSPORT BY VARIOUS INHIBITORS

Additions (100 mμmoles)	NADH-oxidase specify activity (μatoms oxygen consumed/min/mg protein)*				Per cent†	
	I		II			
	0	+TMPD	0	+TMPD	0	+TMPD
None	0·896		0·821		100	
NDGA	0·110	0·718	0·093	0·642	11–12	78–80
Rotenone	0·076	0·600	0·059	0·524	7–9	64–67
Antimycin	0·110	0·541			12	60
Cyanide	0·161	0·161	0·144	0·169	17–18	18–20
HOQNO‡	0·101	0·456			11	50

* Each flask contained from 0·53 to 1·0 mg of protein.
† Per cent of uninhibited controls.
‡ 2-*N*-heptyl-4-hydroxyquinoline-*N*-oxide.

TABLE 4. EFFECT OF NOR-DIHYDROGUAIARETIC ACID (NDGA) ON BEEF HEART MITOCHONDRIAL COENZYME Q–CYTOCHROME *c* REDUCTASE SYSTEM

Additions	Specific activity*		Per cent†
	I	II	
0	40·8	27·1	100
NDGA‡	38·1		93·4
Antimycin§		0·7	2·6

* μmoles Cytochrome *c* reduced per min per mg of protein.
† Per cent of uninhibited controls.
‡ 150 mμmoles per mg protein.
§ 125 mμmoles per mg protein.

Since NDGA inhibits both NADH-oxidase and succinoxidase systems but not cytochrome oxidase or the coenzyme Q–cytochrome *c* reductase system, it is likely that the site of action of NDGA is in complexes I and II (NADH–coenzyme Q reductase and succinate–coenzyme Q reductase systems respectively). This postulation requires further investigation for verification. These studies directly implicate NDGA in the inhibition of mitochondrial electron transport which is consistent with the findings of Burk and Woods[2] concerning NDGA and the inhibition of tumor respiration. Their observation concerning the inhibition of tumor glycolysis by NDGA is more difficult to explain on the basis of electron transport inhibition. They reported that tumor anaerobic glycolysis was inhibited by NDGA and that the addition of NAD^+ or pyruvate reversed the inhibition. These findings suggested that NDGA produced a depressed cellular NAD^+/NADH ratio in their tumor preparations.[2]

Since NDGA had little or no affect on lactate dehydrogenase (L-lactate: NAD oxidoreductase, EC 1.1.1.27),[2] the mechanism involved in the depression of tumor $NAD^+/NADH$ ratio by NDGA remains to be explained. Inhibition of the tumor electron transport system could prevent the coupling of the oxidation of cytoplasmic pyridine nucleotides to mitochondrial oxidative phosphorylation via the glycerophosphate–dihydroxyacetone phosphate or malate–oxalacetate shuttle systems. Since the activity of glycerol-3-phosphate dehydrogenase (L-glycerol-3-phosphate: NAD oxidoreductase; EC 1.1.1.8) in various tumor preparations was low in relation to normal tissues,[9] this possibility appears to be of minor consequence and would not account for the observation that NDGA inhibited tumor anaerobic glycolysis.[2] Another plausible explanation could be that NDGA, in addition to its inhibition of mitochondrial electron transport, induces the transfer of reducing equivalents from the mitochondria to the cytoplasm in sufficient quantity to inhibit tumor glycolysis *in vitro*. The transfer of reducing equivalents has been studied in normal cells.[10] These various possibilities are currently under investigation in our laboratory.

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