Effects of Organic Nitrates on Mitochondrial Respiration and Swelling: Possible Correlations with the Mechanism of Pharmacologic Action

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SUMMARY

Several organic nitrates (e.g., mannitol hexanitrate and erythrityl tetranitrate), were found to induce swelling of rat liver mitochondria *in vitro* and to inhibit the mitochondrial swelling produced by inorganic phosphate and β-hydroxybutyrate. Low concentrations of organic nitrates which are potent vasodilators stimulate oxygen consumption and cause loss of respiratory control of tightly coupled rat liver mitochondria. With organic nitrates of moderate to low pharmacologic activity high concentrations are required to produce loss of respiratory control. The organic nitrates tested which are inactive as coronary dilators do not uncouple oxidative phosphorylation even at very high concentrations. Higher concentrations of the therapeutically employed organic nitrates are required to produce loss of respiratory control with rat heart mitochondria than with liver mitochondria. There is a correlation between (a) the ability of organic nitrates to stimulate mitochondrial respiration, (b) the rate of reaction with reduced glutathione in the presence of liver organic nitrate reductase, and (c) their oil/water partition coefficients.

INTRODUCTION

The known reactivity of organic nitrate vasodilators with reduced glutathione,[1] both nonenzymically and in the presence of liver organic nitrate reductase (1, 2), has stimulated investigation of the possibility that the pharmacologic action of these compounds might involve either direct or enzymic reaction with —SH groups. Although other reactions within the cell have not been ruled out, it seems probable that the action might involve reaction with —SH groups in membranes, with resultant changes in permeability, ion transport, and electrical behavior.

For the initial phase of the investigation mitochondria were chosen as a model membrane system because they are known to be rich in —SH groups. Substances which react with —SH groups, such as iodoacetamide, *p*-chloromercuribenzoate, silver, and mercury, are effective in producing mitochondrial swelling. This suggests that —SH groups play a role in determining mitochondrial structure or permeability (3, 4). In addition, —SH groups are known to be essential for oxidative phosphorylation (5) and for electron transport (6). The uncoupling of phosphorylation by mannitol hexanitrate (7) may result from reaction with —SH groups. A final important consideration in the choice of

[1] The following abbreviations are used: reduced glutathione (GSH), glyceryl trinitrate (GTN), glyceryl dinitrate (GDN), glyceryl mononitrate (GMN), mannitol hexanitrate (MHN), erythrityl tetranitrate (ETN), pentaerythritol tetranitrate (PETN), pentaerythritol trinitrate (PETRIN), isosorbide dinitrate (ISD), inorganic phosphate (P), β-hydroxybutyrate (BOHB or B).

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mitochondria as a test object was the fact that they contain only traces of GSH-organic nitrate reductase, so the interaction of organic nitrates with the membrane system can be studied without the complications of drug transformation by this reaction (8).

This communication reports the effect of organic nitrates on mitochondrial swelling, respiratory control, phosphorylation, and the rate of oxygen uptake.

METHODS AND MATERIALS

Isolation of liver mitochondria. Rat liver mitochondria were isolated by the previously described procedure (9) with 0.33 M sucrose, 0.1 mM EDTA, and 1 mg/ml bovine serum albumin. The final wash and resuspension were made in 0.175 M KCl containing 0.025 M Tris (pH 7.4) for swelling experiments and in 0.33 M sucrose containing Tris for oxygen consumption experiments.

Isolation of heart mitochondria. Rat heart mitochondria were isolated according to the Davis (10) modification of the method of Chance and Hagihara (11). Four to six rat hearts were homogenized in a stainless steel homogenizer with five volumes of cold medium containing 0.25 M sucrose, 0.01 M EDTA, 0.01 M Tris HCl (pH 7.5), and 0.5 mg/ml of *Bacillus subtilis* proteinase. The mitochondria were washed and suspended in 0.25 M sucrose containing 0.01 M EDTA.

Measurement of mitochondrial swelling. Swelling of liver mitochondria was measured by the decrease in absorbance of a dilute suspension at 520 mμ in a Bausch and Lomb Spectronic 20 spectrophotometer at 25°. A decrease in absorbance or light scattering is accepted as an indication of mitochondrial swelling (12). Although the organic nitrates are water soluble at the final concentrations used, concentrated stock solutions cannot be prepared in water. Therefore, appropriate aliquots of organic nitrates in alcohol were placed in the tubes and the solvent was driven off with a stream of air. Then the medium, 0.33 M sucrose containing 0.025 M Tris pH 7.4, was added and 10 minutes allowed for the nitrates to dissolve. After any additional substances had been added, the experiment was started by the addition of mitochondria. Absorbancy readings were taken at 1-minute intervals for the first 10 minutes, and at 5-minute intervals thereafter.

Measurement of oxygen consumption. Oxygen consumption was followed with a Beckman Oxygen Macro Electrode (325-814), using a Beckman Physiological Gas Analyzer (Model 160) and a Varian Dual Channel Recorder (G22A). All experiments were performed at 25°. Controls were run simultaneously with the nitrate treated mitochondria. When organic nitrates were used they were added to the cuvette and the organic solvent was removed with a stream of air before the medium was added. The incubation medium for measurement of oxygen consumption with liver mitochondria consisted of 20 mM potassium phosphate (pH 7.5), 10 mM $MgCl_2$, 0.1 mM EDTA, 10 mM NaF, and 200 mM sucrose. For heart mitochondria the medium contained 10 mM potassium phosphate (pH 7.5), 10 mM KCl, 0.25 mM EDTA, and 250 mM sucrose.

The concentration of the ADP used was standardized enzymically by measuring the disappearance of DPNH spectrophotometrically (340 mμ) in a system which couples the reactions catalyzed by pyruvate kinase and lactate dehydrogenase (13).

Determination of oil/water partition coefficients. The oil/water partition coefficient was determined by a modification of the method described by Bell *et al.* (14). A known amount of organic nitrate was equilibrated between equal volumes of cottonseed oil and water. The total nitrate level in the aqueous phase was then assayed with phenoldisulfonic acid (15).

Materials. Biochemical reagents were obtained from Sigma Chemical Company, St. Louis. Organic nitrates were gifts of the Atlas Chemical Industries, Inc., Wilmington, Delaware, and Propellex Chemical Company, Edwardsville, Illinois. Glyceryl dinitrate was prepared enzymically from glyceryl trinitrate and isolated by prepara-

tive thin-layer chromatography (8). *Bacillus subtilis* proteinase was obtained as Nagarse from the Enzyme Development Corporation, New York. Other chemicals were of analytical reagent grade. All aqueous solutions were prepared in water redistilled in a two-step all quartz still.

RESULTS

Effect of Organic Nitrates on Mitochondrial Swelling

Induction of swelling. Figure 1 illustrates the effect of various organic nitrates on the rate of swelling of liver mitochondria in the absence of exogenous substrate. Glyceryl trinitrate (GTN), glyceryl mononitrate (GMN), isosorbide dinitrate (ISD), and pentaerythritol trinitrate (PETRIN) in concentrations up to 0.5 to 1 mM did not affect the rate of mitochondrial swelling in the KCl-Tris medium, but mannitol hexanitrate (MHN), erythrityl tetranitrate (ETN), pentaerythritol tetranitrate (PETN), and glyceryl dinitrate (GDN), produced gradual swelling. The concentrations necessary to produce these effects were high (0.2–0.8 mM) in most cases, except for MHN (0.09 mM).

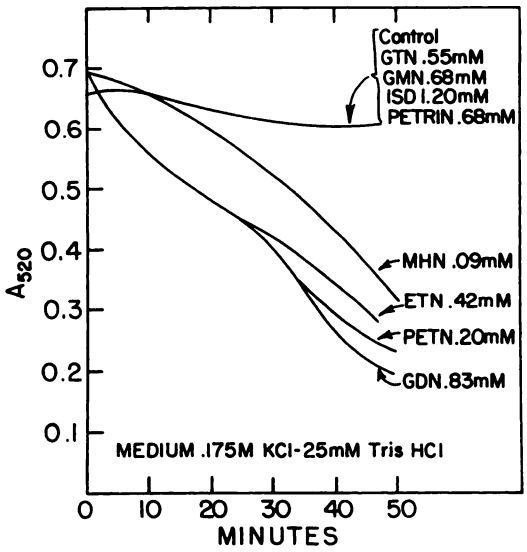

FIG. 1. *The effect of organic nitrates on the absorbance of dilute suspensions of rat liver mitochondria in the absence of exogenous substrate*

A decrease in the A_{520} reading is an indication of mitochondrial swelling. The incubations were carried out at 25° in KCl-Tris medium. The concentrations used are indicated in the figure.

Swelling Induced by Inorganic Phosphate and β-Hydroxybutyrate

Mitochondrial swelling is induced by inorganic phosphate and a substrate such as β-hydroxybutyrate (BOHB) in the absence of a phosphate acceptor (9, 12). The rate of swelling is related to the rate of electron transport and phosphorylation (16). Uncoupling agents in concentrations which completely eliminate the phosphorylation of ADP generally block mitochondrial swelling (17). MHN, GDN, and PETN decreased the rate of swelling produced by phosphate and BOHB (P + B), whereas ETN accelerated the initial rate but decreased the extent of swelling. GTN, GMN, ISD, and PETRIN showed no inhibition of swelling (Fig. 2). Those agents which were effective in decreasing and/or delaying swelling induced by P + B were the same ones which were active in producing swelling of liver mitochondria in the absence of added phosphate and substrate as shown in Fig. 1. MHN, GTN, and ETN have been shown previously to uncouple oxidative phosphorylation in liver mitochondria (7). The inhibition of swelling induced by P + B could be the result of a number of mechanisms, such as inhibition of electron transport, uncoupling activity, or reaction with high energy intermediates.

Effect of Organic Nitrates on Mitochondrial Respiration

Rat liver mitochondria. Figure 3 presents a tracing which illustrates the respiration of rat liver mitochondria as measured with the oxygen electrode. The initial concentration of oxygen in the air saturated medium is 240 μM (18). The addition of mitochondria causes an abrupt fall in oxygen tension owing to a dilution of the oxygen present by the addition of the essentially anaerobic stock suspension of mitochondria. The addition of BOHB to the mitochondria produces a moderate increase in the rate of respiration. The rate

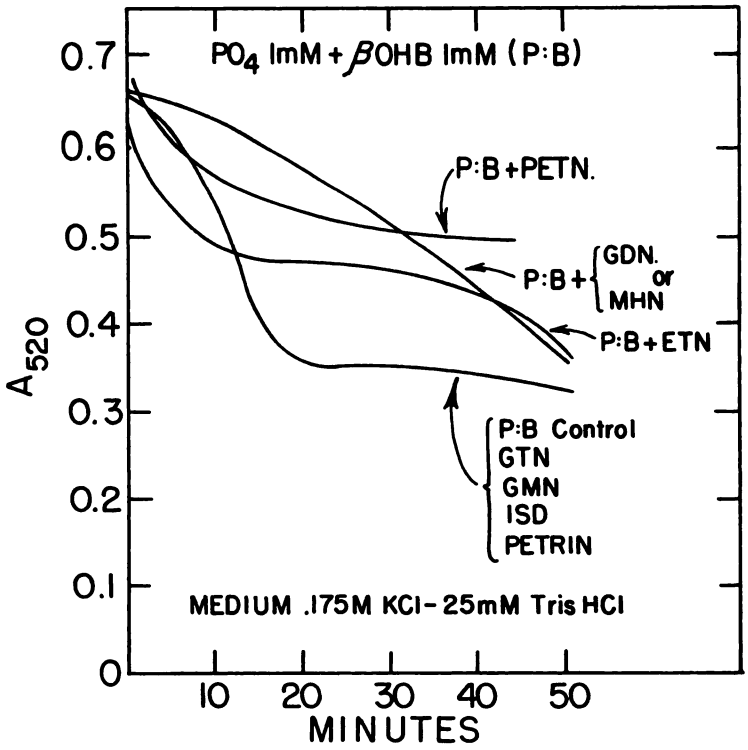

FIG. 2. *The effect of organic nitrates on phosphate and β-hydroxybutyrate-induced swelling of rat liver mitochondria*

Except for the addition of inorganic phosphate and β-hydroxybutyrate, the conditions and the concentrations of the organic nitrates were similar to those for the experiment shown in Fig. 1.

of oxygen uptake is greatly accelerated by addition of the phosphate acceptor ADP. A constant rate of oxygen uptake is maintained until all the ADP is phosphorylated to ATP, and then the rate returns to the original value with substrate.

The respiratory control ratio, i.e., the rate of oxygen uptake in the presence of added ADP divided by the rate of oxygen uptake in the absence of ADP, is 4.0 in the experiment shown in Fig. 3. Tightly coupled mitochondria have high respiratory control ratios, whereas loosely coupled mitochondria have little or no dependence of respiratory rate on ADP. A high respiratory control ratio is an indication of the integrity of mitochondria structure and phosphorylation mechanisms.

The ADP:O ratio is calculated as the ratio of the added ADP to the additional oxygen utilized during the phosphorylation of the ADP. In this experiment the ADP:O ratio was 2.8. The ADP:O ratio in tightly coupled mitochondria is equivalent to the P:O ratio (18).

GTN has no effect on the oxygen uptake in the absence of added substrate (Fig. 3). When BOHB is added to mitochondria in the absence of GTN, there is some increase in the rate of oxygen uptake (control). However, with 530 μM GTN present, the addition of BOHB results in a fourfold increase in the rate of oxygen consumption over that in the control. The rate of oxygen consumption in the presence of GTN is equal to the rate after ADP is added to the control. This stimulation of oxygen consumption by GTN suggests an uncoupling effect.

The 530 μM GTN causes a complete loss of respiratory control as indicated by no change in the rate of oxygen consumption upon the addition of ADP. In the presence of ADP the ratio of the rate of oxygen

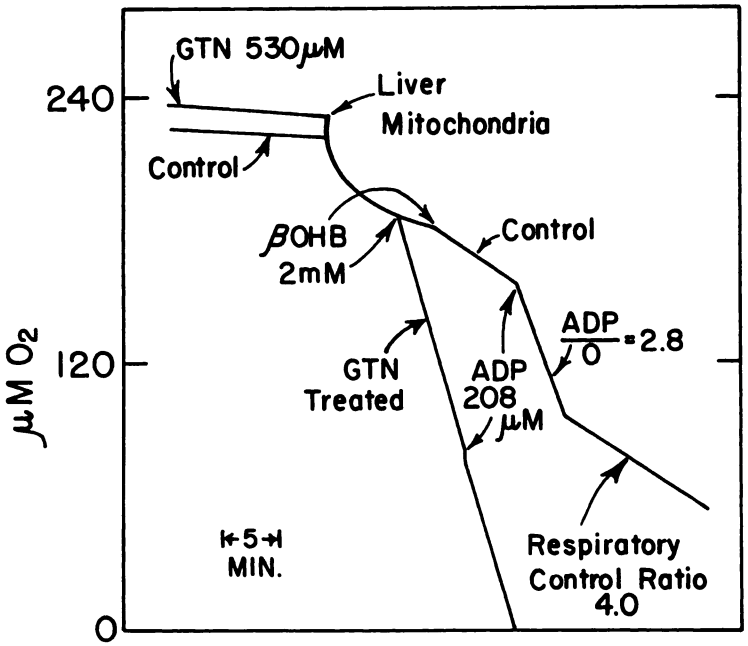

FIG. 3. *The effect of glyceryl trinitrate on the oxygen consumption by rat liver mitochondria*

The incubations were carried out at 25° in a medium containing: 20 mM potassium phosphate, 10 mM magnesium chloride, 0.1 mM EDTA, 10 mM sodium fluoride, and 200 mM sucrose. The ordinate shows the oxygen concentration (μM) in the medium. The mitochondrial concentration was approximately 1 mg protein per milliliter. The concentrations and time of addition of various substances are indicated in the figure.

consumption by the GTN-treated mitochondria to the control was 1.0, emphasizing that GTN induced a response equal to the enhanced rate of oxygen consumption seen with ADP alone. Release of mitochondria from respiratory control is an indication of uncoupling activity (18).

Table 1 presents structure-activity and dose-response studies of the effect of organic nitrates on respiratory control of rat liver mitochondria. The compounds are listed in the order of decreasing activity for producing complete loss of respiratory control. The first five nitrates in this list are widely employed therapeutically in the treatment of angina pectoris, and low to moderate concentrations (27–1500 μM) are required to produce complete loss of respiratory control. The next three compounds, which are less active pharmacologically, are required in higher concentrations (4.0–6.7 mM). Finally, GMN and potassium nitrate, which are inactive as coronary dilators, cannot uncouple oxidative phosphorylation even at very high concentrations (16.7–33.4 mM). The concentrations required to produce minimal detectable stimulation of mitochondrial respiration is considerably lower (0.67–250 μM) for the therapeutically employed agents.

The stimulation of respiration by the organic nitrates is indicated by the ratio of the rate with nitrate to the rate in the control (both in the presence and in the absence of ADP). In general there is a twofold stimulation (Table 1). The highest response occurred with GTN, which produced a fourfold increase in respiration, as shown also in Fig. 3. This response is dependent on added substrate, as organic nitrates had no effect on respiration with mitochondria alone. A nitrate to control ratio of 1.0 for the rate of respiration in the presence of ADP indicates no further increase in oxygen consumption upon the addition of ADP. Ratios of less than 1.0 indicate some inhibition of the rate of oxygen uptake in the presence of ADP.

TABLE 1

Effect of organic nitrates on respiratory control in rat liver mitochondria

The medium employed is shown under Fig. 3. Abbreviations are listed in footnote 1. The rate of oxygen consumption in the presence of the concentration of organic nitrate which gives maximal stimulation when added alone (column 1) is compared to controls both in the absence and in the presence of ADP. The last column shows the range of respiratory control indices for all of the mitochondrial preparations used.

Nitrate	Conc. for complete loss of respiratory control		Conc. for minimal detectable stimulation of respiration	O_2 consumption nitrate: control at maximal response		Respiratory control index (untreated) mitochondria
	μM	Relative potency		−ADP	+ADP	
MHN	27	19.6	0.67 μM	2.7	1.0	2.5–3.4
ETN	270	2.5	13 μM	2.4	1.0	3.9–4.3
PETN	500	1.1	100 μM	1.3	0.3	4.2–5.0
GTN	530	1.0	67 μM	3.9	1.0	3.1–4.0
ISD	1500	0.35	250 μM	1.8	0.5	4.3–6.0
1,2-GDN	4000	0.13	2.0 mM	1.0	0.2	4.0–5.5
PETRIN	4200	0.12	2.1 mM	1.6	0.7	4.5–5.1
$NaNO_2$	6700	0.08	3.2 mM	2.2	1.0	3.5–4.0
GMN	No effect at 16.7 mM	—	—	—	—	3.8–4.7
KNO_3	No effect at 33.4 mM	—	—	—	—	4.0–4.3

The relative potency of the nitrates indicated in Table 1 is calculated on the basis of the concentration required to produce complete loss of respiratory control, with GTN set arbitrarily at 1.0. The last column in Table 1 represents the respiratory control indices for all mitochondrial preparations used. They fall in the general range of 3–6, indicating that the mitochondria were tightly coupled and therefore dependent on the phosphate acceptor ADP to show their maximal respiratory rate. In view of the findings with liver mitochondria, rat heart mitochondria were examined, for the possibility existed that heart mitochondria would be more sensitive or show a different response.

Rat heart mitochondria. Figure 4 presents a tracing of the oxygen consumption by isolated rat heart mitochondria. Glutamate was used as the substrate since it gave higher levels of respiratory control than BOHB or succinate. The tight coupling of phosphorylation to electron transport in these heart mitochondria is indicated by the respiratory control ratio of 10 and an ADP:O ratio of 3.1. When heart mitochondria are incubated in this medium,

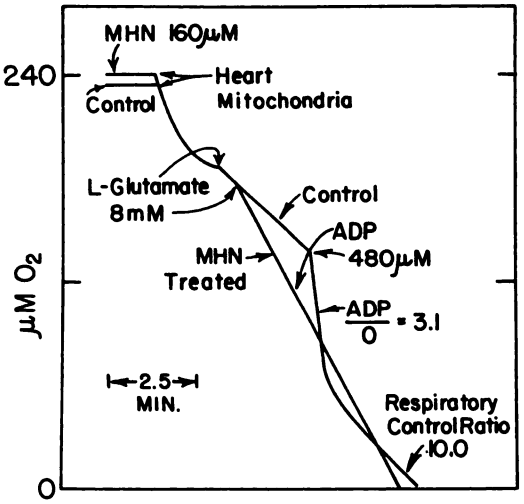

FIG. 4. *The effect of mannitol hexanitrate on the oxygen consumption by rat heart mitochondria*

The incubations were carried out at 25° in a medium containing: 10 mM potassium phosphate (pH 7.5), 10 mM Tris HCl (pH 7.5), 10 mM potassium chloride, 0.25 mM EDTA, and 250 mM sucrose. The concentrations and time of addition of various substances are indicated in the figure.

there is no indication of ATPase activity, as the addition of ATP causes no stimulation of oxygen consumption. If ATP were

TABLE 2

Effect of organic nitrates on respiratory control of rat heart mitochondria

The medium employed is shown under Fig. 4. All abbreviations and column headings are as in Table 1.

Nitrate	Conc. for complete loss of respiratory control		Conc. for minimal detectable stimulation of respiration	O_2 consumption nitrate: control at maximal response		Respiratory control index (untreated) mitochondria
	μM	Relative potency		−ADP	+ADP	
MHN	160	5.0	1.7 μM	2.0	0.2	7.4–10
PETN	670	1.2	67 μM	2.5	0.6	6.8–9.2
GTN	800	1.0	220 μM	2.7	0.7	8.0–10
ETN	1,070	0.75	206 μM	3.0	0.6	8.3–10.5
PETRIN	2,080	0.38	400 μM	2.3	0.3	6.0–7.0
1,2-GDN	2,500	0.32	1.25 mM	1.9	0.5	6.0–10
ISD	4,400	0.18	1.53 mM	2.0	0.2	9.0–11
$NaNO_2$	16,700	0.04	2.20 mM	2.0	0.6	7.0–8.4
GMN	No effect at 21.7 mM	—	—	—	—	7.0–8.6
KNO_3	No effect at 28.6 mM	—	—	—	—	6.0–8.5

hydrolyzed to ADP, the oxygen uptake would be increased.

The presence of MHN at 160 μM stimulated the respiration in presence of glutamate, producing a twofold increase in rate (nitrate:control ratio in absence of ADP = 2). When ADP is added there is no change in rate. This indicates complete loss of respiratory control. The rate of respiration of heart mitochondria with MHN and ADP present is considerably less than the rate observed with ADP alone. In fact, the ratio of the respiratory rate for nitrate: control in the presence of ADP is only 0.2.

Table 2 presents the effects of various organic nitrates on rat heart mitochondria. With the therapeutically employed nitrates higher concentrations are required to produce minimal detectable stimulation of respiration and complete loss of respiratory control in heart mitochondria than in liver mitochondria. There is some rearrangement in the order of potency, but the lack of response to the therapeutically inactive nitrates still is apparent. The nitrates cause a two- to threefold increase in the rate of oxygen uptake in the absence of ADP. In the presence of ADP the ratio for the rate of oxygen uptake in the nitrate treated as compared to the control is less than 1.0, indicating an inhibition of the mitochondrial respiration in the presence of ADP. This effect was seen in all cases with heart mitochondria, but only in some cases with liver mitochondria. This response may be the result of inhibitory effects of organic nitrates on electron transport and/or phosphorylation enzymes in addition to the uncoupling activity responsible for release from respiratory control.

The relative potencies indicated in Table 2 are based on comparison of the concentrations required to produce complete loss of respiratory control, with GTN set equal to 1.0. The high respiratory control indices in the untreated controls, as summarized in the last column of the table, indicate that the heart mitochondria were tightly coupled.

Correlation between Organic Nitrate Effects

Table 3 presents possible correlations between various organic nitrate effects. The first column of data represents the ratio of the concentrations necessary to produce complete loss of respiratory control in heart mitochondria as compared to liver mitochondria. The therapeutically employed nitrates are required at 1.3- to 5.9-fold higher concentrations to produce loss

TABLE 3

Correlation between various organic nitrate effects

The abbreviations employed are as in footnote 1. The ratio of the concentration needed to produce maximal stimulation of respiration in heart mitochondria as compared to liver mitochondria is calculated from the values presented in Tables 1 and 2. The V_{max} values with liver GSH-organic nitrate reductase were obtained from a previous publication (2). The oil/water partition coefficient represents the ratio of nitrate concentrations after equilibration between equal volumes of cottonseed oil and quartz-distilled water. PETN was insoluble in both phases, whereas $NaNO_2$ and KNO_3 were insoluble in the oil phase.

Nitrate	Conc. for maximal stimulation of respiration (heart:liver ratio)	Conc. for loss of respiratory control, liver		V_{max}, liver GSH-organic nitrate reductase		Oil/water partition coefficient
		μM	Relative potency	mmole/kg/min	Relative potency	
MHN	5.9	27	19.6	830	6.9	486
ETN	4.0	270	2.5	358	3.0	326
PETN	1.3	500	1.1	1.4	—	Insoluble
GTN	1.5	530	1.0	120	1.0	115
ISD	2.9	1500	0.35	21.5	0.18	41
1,2-GDN	0.6	4000	0.13	5.0	0.04	—
PETRIN	0.5	4200	0.12	9.1	0.08	24
$NaNO_2$	2.5	6700	0.08	—	—	0
GMN	—	No effect at 16.7 m*M*	—	0.7	<0.01	2.8
KNO_3	—	No effect at 33.4 m*M*	—	—	—	0

of respiratory control in heart mitochondria.

The next three sections of the table indicate that there is a definite correlation between (a) the ability of nitrates to stimulate respiration, (b) the rate of reaction with GSH in the presence of GSH-organic nitrate reductase, and (c) their oil-water partition coefficients. The comparisons are based on the maximal effects with liver mitochondria. In all these studies, the water-soluble $NaNO_2$, GMN, and KNO_3 show very low or no activity. The alkyl organic nitrate esters with the higher oil-water coefficients show greater potency in affecting mitochondrial respiration and a more rapid enzymic denitration. PETN is extremely insoluble in aqueous media, so its rate of denitration in the presence of GSH organic nitrate reductase could not be determined definitively.

DISCUSSION

There appears to be no direct relationship between the effect of organic nitrates in producing changes in mitochondrial volume and structure and their ability to stimulate oxygen consumption in rat liver or heart mitochondria. MHN, ETN, PETN, and GDN produced swelling in the absence of substrate and partially inhibited the swelling produced by phosphate in the presence of BOHB, whereas GTN, GMN, ISD, and PETRIN had no effect in either case. On the other hand, all these organic nitrates except GMN were capable of stimulating oxygen consumption at much lower concentrations than those employed in the swelling experiments. Even when swelling is produced the time course is too slow to be consistent with the rapid onset of the therapeutic effect of the organic nitrates.

The magnitude of the stimulation of respiration by organic nitrates is similar for heart and liver mitochondria, but higher concentrations of nitrates are required to produce maximal effects with heart mitochondria. Another difference is the more consistent inhibition by the ni-

trates of the maximal rate of respiration observed in the presence of ADP in the case of heart mitochondria. These differences between rat heart and rat liver mitochondria in the response of the respiration to organic nitrates do not appear to be related to the absence of Mg^{++} from the incubation medium used with heart mitochondria. The heart mitochondria have a very high ATPase activity in the presence of 0.1 mM Mg^{++}, whereas the liver mitochondria did not exhibit ATPase activity in the presence of 10 mM Mg^{++}. However, the addition of Mg^{++} did not enhance the stimulation of oxygen consumption by MHN with heart mitochondria. The presence of MHN did not affect the stimulation of heart mitochondrial ATPase by 0.1 mM Mg^{++}.

Krantz *et al.* (19) found that GTN, amyl nitrite, and octyl nitrite diminished ATPase activity in homogenates of rabbit aorta, whereas sodium nitrate and sodium nitrite were ineffective. GTN and amyl nitrite had no effect on skeletal muscle or uterine smooth muscle ATPase. Carr *et al.* (20), studying the effect of GTN and octyl nitrite on the ATPase of homogenates of dog coronary artery, failed to demonstrate any marked inhibition.

The effect of organic nitrates on the metabolism of arterial strips was studied with rat aorta by Krantz *et al.* (21). They found that low concentrations of GTN and sodium nitrite partially inhibited the oxygen consumption of the arterial tissue, whereas higher concentrations caused no further change or actually increased oxygen uptake. ETN, octyl nitrite, amyl nitrite, and sodium nitrate did not inhibit the oxygen uptake of the arterial tissue. Brahen and Krantz (22), found that sodium nitrite and amyl nitrite depressed respiratory metabolism in beef coronary arteries but sodium nitrate did not.

A comparison of the relative potency of organic nitrates in ability to cause release from respiratory control in mitochondria to the oral therapeutic dose in man shows no quantitative relationship. MHN, ETN, PETN, ISD, and $NaNO_2$, which differ greatly in their ability to release respiratory control, are used in oral doses of 20–60 mg. Other nitrates are administered sublingually in lower doses. However, the therapeutic responses are by no means equal. The difficulties in appraising the clinical effectiveness and the use of different routes of administration make quantitative comparisons extremely difficult. The fact remains that therapeutically inactive nitrates do not affect mitochondrial respiration. Relatively high concentrations are required with nitrates which are moderately effective pharmacologically, whereas the therapeutically employed nitrates produce effects in considerably lower concentrations.

These correlations present two possibilities: (a) The first would be that the clinical effectiveness of these agents is related to release from respiratory control in mitochondria (presumably due to their uncoupling activity). For detectable stimulation of oxygen consumption moderately low concentrations were effective. How release from respiratory control or partial uncoupling might lead to the therapeutic response is unknown. Complete uncoupling would upset the metabolic efficiency of the heart. (b) A second and more likely possibility is that the correlation in these results may merely be an expression of some parallel reaction mechanism or property of the compounds that is necessary to produce both loss of respiratory control and relief of the anginal syndrome. The reactivity of organic nitrates with sulfhydryl groups may be of great importance for the mechanism of their pharmacologic action, but prime effect may be through sulfhydryl groups other than those in mitochondria. Such reactions might affect the ability of membranes to determine the distribution of substrates, the concentrations of ions, and general control of metabolism. There is also the possibility that organic nitrates affect sulfhydryl groups in contractile proteins.

There is a relationship between (a) the ability of organic nitrates to stimulate respiration of mitochondria, (b) the rates of reaction with GSH in the presence of GSH-organic nitrate reductase, and (c)

their oil/water partition coefficients. The reason why the more lipid soluble organic nitrates show a more rapid reaction with organic nitrate reductase or with mitochondria is unknown. Mitochondria do exhibit many permeability characteristics similar to other lipid containing membranes and probably favor the entry of lipid-soluble materials in agreement with the known high lipid content of the membrane (23). The potency of dinitrophenol analogs as uncouplers of oxidative phosphorylation in mitochondria also shows a correlation with their oil/water partition coefficients (24).

The ability of organic nitrates to affect mitochondrial respiration does not appear to be mediated by inorganic nitrite ion, since added nitrite ion is very much less active than the intact organic nitrate esters. It seems highly unlikely that the stimulation of respiration is due to intramitochondrial formation of nitrite ion by reaction of organic nitrates with GSH, since the denitrating enzyme is located in the soluble fraction of the cell and the GSH level of the washed mitochondria is negligible.

ACKNOWLEDGMENT

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