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Effect of organic nitrates on myocardial oxygen consumption *in vitro*

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Summary

1. Mannitol hexanitrate (MANHN), iditol hexanitrate and sorbitan tetranitrate, all at 442 μM, stimulate oxygen uptake by isolated rabbit atrial tissue.

2. At the same concentration, glyceryl trinitrate decreased oxygen uptake, while isosorbide dinitrate and erythrityl tetranitrate had no effect.

3. No simple correlation was found between the lipoid solubility or per cent nitration of these compounds and their effects on oxygen uptake.

4. The stimulating effect of MANHN and its possible role as an uncoupler of oxidative phosphorylation is discussed.

Introduction

In recent years there has been increased emphasis on the direct and indirect myocardial actions of organic nitrates to explain their therapeutic actions in man (Rowe, 1968). While many of the data in animals and man point to the fact that nitrates are capable of reducing myocardial oxygen consumption (Rowe, 1968), the direct cellular mechanism of this effect, if any, is not completely understood. Moreover, little information is available comparing the actions of a variety of organic nitrates on cardiac muscle metabolism.

In order to provide further information on the basic actions of organic nitrates on cardiac metabolism, the following problems were studied: (*a*) the direct effects of various organic nitrates on basal myocardial oxygen uptake (qO_2); (*b*) the structure-activity-relations for these agents in terms of their effects on myocardial oxygen consumption.

Methods

The oxygen uptake (qO_2) of isolated rabbit left atria was measured by the direct Warburg method, utilizing an automated manometer apparatus which permitted continuous direct recording of O_2 uptake (Levy & Richards, 1964 ; 1965). Female albino rabbits (2·0–2·5 kg) were killed, exsanguinated, and their hearts quickly removed. The left atrial appendage was dissected from the rest of the heart and sectioned before placing in Warburg flasks. The flasks contained 2·8 ml of a Krebs-Ringer phosphate buffer (Levy & Richards, 1965) containing 5·5 mM glucose as substrate. Drug and solvent effects were studied by adding appropriate amounts of the agent to the stock buffer solution to yield the desired final molar concentration in the flask. The buffer solution was gassed with 100% O_2 before placing it in

the flask. The centre well contained 0·2 ml of 20% KOH. After putting the tissue in the flasks containing the buffer solution, they were mounted on the manometers and gassed with 100% O_2 for 2 min while being shaken in a 37·5° C constant temperature tank. At the end of this equilibration period, the flasks were sealed and O_2 uptake continuously recorded for 180 min. At the end of the experiment, the tissues were removed, blotted on filter paper, and dried in an oven for 24 h for determination of dry weights. The data are presented either as qO_2 (μl O_2 consumed/mg dry weight per h) or plotted as μl O_2 consumed/mg dry weight against time.

The nitrates used were supplied in aqueous or propylene glycol solutions. The former solutions could be used for some drugs when low final flask concentrations were needed. Results from these groups may be compared with untreated controls (unless otherwise specified). For those agents in propylene glycol that required appropriate solvent controls, three different solvent volumes were studied. Thus some agents are compared with untreated controls ; others are compared with the appropriate solvent control group.

Statistical significance between various groups was determined by Student's *t* test for uncorrelated means. Significant differences were defined when *P* was 0·05 or less.

Results

Table 1 summarizes the effects of various organic nitrates and propylene glycol solvent on atrial O_2 consumption in a glucose-fortified Krebs-Ringer buffer. A significant decrease in O_2 uptake was observed with a 442 μM concentration of glyceryl trinitrate (GTN). In a similar concentration, mannitol hexanitrate

TABLE 1. *Effects of organic nitrates on O_2 uptake of rabbit atrial muscle (glucose buffer)*

Compound	Conc. (μM)	*N*	Control group comparison	qO_2* 60 min	*P*	120 min	*P*	180 min	*P*
Glyceryl trinitrate	442	12	*vs.* II	6·05±0·19	0·02	5·11±0·17	0·001	4·72±0·14	0·001
Mannitol hexanitrate	4·42	13	*vs.* I	7·04±0·32	NS	6·41±0·33	0·05	6·14±0·27	0·01
Mannitol hexanitrate	442·0	6	*vs.* II	11·72±0·93	0·01	11·60±0·84	0·01	10·96±0·77	0·001
Sorbitan tetranitrate	442·0	9	*vs.* III	7·93±0·32	NS	7·43±0·17	0·02	7·07±0·15	0·05
Iditol hexanitrate	442·0	8	*vs.* IV	12·3 ±0·75	0·001	10·52±0·61	0·01	9·24±0·37	0·01
Isosorbide dinitrate	442·0	15	*vs.* III	6·81±0·22	NS	6·06±0·19	NS	5·48±0·19	NS
Erythrityl tetranitrate	442·0	9	*vs.* III	8·00±0·43	NS	6·93±0·33	NS	6·57±0·36	NS
Controls	(μl/ml)								
I Untreated controls	—	11	—	6·41±0·31	—	5·47±0·24	—	5·00±0·23	—
II Propylene glycol	2·5	9	*vs.* I	7·02±0·33	NS	6·72±0·30	0·01	6·49±0·31	0·001
III Propylene glycol	7·4	6	*vs.* I	7·01±0·41	NS	6·30±0·38	NS	5·94±0·44	NS
IV Propylene glycol	22·3	9	*vs.* I	8·29±0·41	0·01	7·91±0·43	0·001	7·47±0·45	0·001

* qO_2 = μl O_2/mg dry wt. per h (mean±S.E.M.).

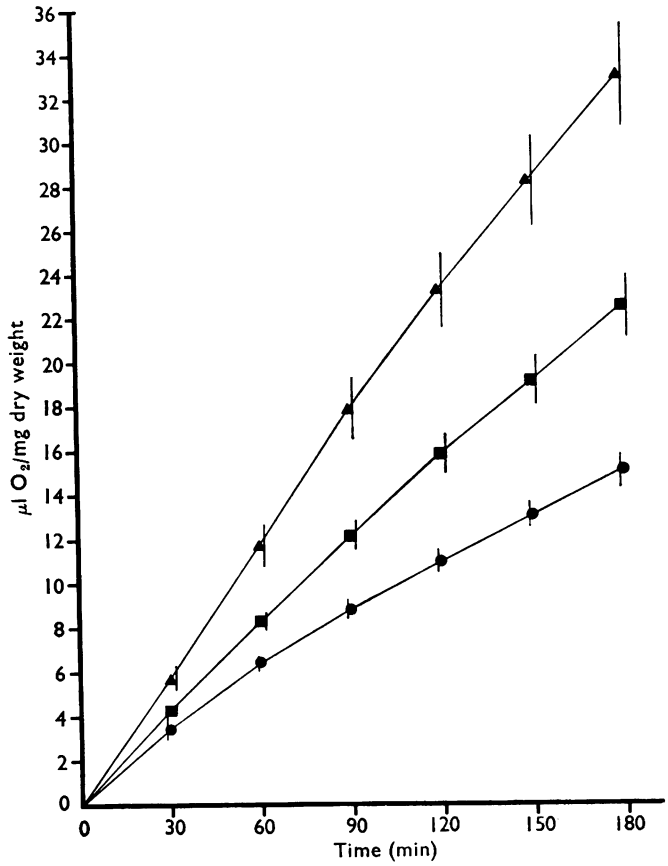

FIG. 1. Effect of 442 μM mannitol hexanitrate (▲) on O_2 uptake of quiescent rabbit atrial muscle compared with untreated (●) and propylene glycol (■) solvent controls. Each point is the mean ± S.E.M. of between six and eleven experiments. Solvent volume = 22·3 μl propylene glycol/ml buffer.

TABLE 2. *Some chemical properties of organic nitrates*

Compound	Mol. wt.	% NO_2	Oil/water partition ratio (K)*
Glyceryl trinitrate	227·1	81·9	77
Iditol hexanitrate	452·2	82·3	†
Mannitol hexanitrate	452·2	82·3	770
Sorbitan tetranitrate	344·1	72·1	1,100
Isosorbide dinitrate	236·1	26·3	28
Erythrityl tetranitrate	302·1	82·0	550

* Ratio calculated from partition data using the relationship: $K = C_1/C_2$, where K is the partition coefficient of the nitrate at 25° C (using equal volumes (50 ml) of the two immiscible solvents, corn oil and H_2O); C_1 = mg of solute in oil phase (determined by weight of sample taken, less weight of nitrate found by analysis in the H_2O phase); C_2 = mg solute in H_2O phase (determined by a 2,6-xylenol colorimetric procedure for nitrate). Sample sizes ranged from 50–300 mg. Equilibration (shaking) time ranged from 1–68 h in order to test whether successively increasing shaking times influenced nitrate partition. Data indicate a constancy of results independent of equilibration time. Standard curve determined from suitable known quantities of potassium nitrate.

† Data not available.

(MANHN), sorbitan tetranitrate (SORBTN) and iditol hexanitrate (IDHN) produced significant increases in atrial qO_2. A stimulation of oxygen consumption was also seen with MANHN at a concentration of 4·42 μM. Isosorbide dinitrate (ISDN) and erythrityl tetranitrate (ERTN) in concentrations of 442 μM had no significant effect on O_2 uptake compared with appropriate controls.

Figure 1 shows the time course of the stimulatory effect of MANHN (442 μM) on O_2 uptake compared with untreated and propylene glycol controls. Figure 2 indicates the lack of effect of ISDN in an equimolar concentration.

Table 2 compares the molecular weights, % nitration and oil/water partition ratio of the agents studied.

Discussion

While data are available dealing with the effects of various nitrates on cellular respiration of vascular smooth muscle, as well as rat liver and heart mitochondrial preparations (Krantz, Carr & Knapp, 1951 ; Needleman & Hunter, 1966), quantitative information on the effects of these agents on basal O_2 uptake of intact myocardial tissue has not been described.

The results presented in this report emphasize two features of the nitrate effect on cardiac muscle oxidative metabolism: (1) important differences were found between various organic nitrates in terms of their effects on myocardial oxygen uptake *in vitro* ; (2) differences in effects on O_2 uptake produced by the compounds do not appear to be related solely to lipoid solubility or % nitration of the molecule.

The most important qualitative difference between the nitrates studied is that MANHN, IDHN and SORBTN, in a concentration of 442 μM, are capable of producing a significant increase in myocardial oxygen uptake in a glucose-fortified

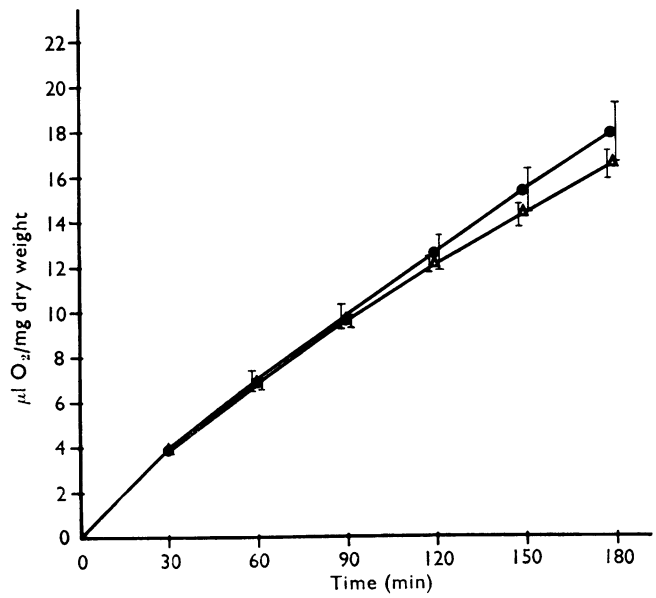

FIG. 2. Effect of 442 μM isosorbide dinitrate (△) on atrial muscle O_2 uptake compared with propylene glycol controls (●). N = Six to fifteen experiments. Solvent volume = 7·4 μl/ml buffer.

Krebs-Ringer buffer. The therapeutically important compound GTN, on the other hand, produced a significant depression of oxygen consumption in the same concentration. Isosorbide dinitrate and ERTN were without significant effect.

This profile of effect on cardiac oxygen consumption basically conforms to the previously described ability of certain of these agents to uncouple oxidative phosphorylation of rat liver and heart mitochondrial preparations (Needleman & Hunter, 1966). Thus, MANHN, which is the most potent of the agents studied here in terms of its stimulant effect on oxygen consumption, also has been shown to be the most active of several nitrates studied with regard to its stimulation of mitochondrial oxygen uptake and inhibition of cellular respiratory control (uncoupling of oxidative phosphorylation) (Needleman & Hunter, 1966). The stimulant effect on O_2 uptake seen in rat heart mitochondria was observed in a glutamate (8 mM) fortified buffer (Needleman & Hunter, 1966).

Unlike classic uncouplers, however, MANHN caused mitochondrial swelling as well as inhibition of dinitrophenol stimulated ATPase (Hunter, Kahana & Ford, 1953 ; Needleman & Hunter, 1966). Nitrates such as GTN, ERTN and ISDN were relatively inactive as uncouplers of oxidative phosphorylation. In addition, in terms of the concentrations of these latter agents needed to produce complete loss of respiratory control of rat heart mitochondria, the concentrations used in the present study would not be expected to produce any stimulant effect on myocardial tissue oxygen uptake. The inhibitory effect of GTN on cardiac O_2 uptake remains to be explained. The O_2 consumption of canine myocardial homogenate preparations is also inhibited by GTN (Bachand, Somani & Hardman, 1969).

Another point of interest of this study concerns the possible structure-activity-relations for these agents in terms of their effects on oxidative metabolism of isolated heart muscle. Two features may be emphasized from the data: (*a*) degree of nitration and (*b*) lipoid solubility.

The data show that no pattern of *in vitro* activity can be correlated with the number of nitrate groups, or percentage nitration of the molecule. This is consistent with other pharmacological effects of these agents (Riseman, Koretsky & Altman, 1965 ; Krantz, Carr, Forman & Cone, 1940).

Similarly, no easily apparent correlation exists between the oil/water partition ratios and the effects on atrial muscle O_2 uptake. Thus, the lipophilic compounds MANHN and SORBTN produced a marked stimulation of tissue O_2 consumption. Erythrityl tetranitrate (ERTN), while having the same order of magnitude of lipoid solubility as MANHN, had no effect in the concentration studied. ISDN, which was also without effect on O_2 uptake, is almost 20 times less lipoid soluble than ERTN. Preliminary experiments with borneol nitrate, an extremely lipoid soluble agent, indicated that it caused a slight inhibition of O_2 consumption.

Therefore, these data do not permit the conclusion that the simple oil/water partition coefficient of any given nitrate is a major determinant or predictor of its ability to stimulate myocardial cellular respiration. While direct comparisons between studies with rat heart mitochondrial preparations and intact atrial muscle are difficult, the noted major exceptions to the postulated importance of nitrate lipoid solubility being correlated with ability to stimulate tissue respiration (Needleman & Hunter, 1966) require that other explanations be considered.

One possibility is that effects on cellular respiration may be a function of some optimal lipid-aqueous solubility ratio, rather than dependent on a linearly proportional relationship between lipoid solubility and biological effect. Thus, nitrate effects on cellular respiration perhaps may be better described by the postulated parabolic relationship between lipoid solubility and biological response as described for other classes of compounds (Hansch, Steward, Anderson & Bentley, 1968). That is, extremes of lipoid or aqueous solubility might preclude attaining optimum biophase-intracellular concentrations required for any given effect.

Finally, it is of interest to note the stimulation of O_2 uptake produced with high concentrations of the propylene glycol solvent. This stimulation of respiration is in contrast to the reported inhibition of O_2 uptake and oxidative phosphorylation of bovine heart mitochondria produced by other polyhydroxyl compounds such as glycerol, ethylene glycol and *n*-propyl alcohol (Conover, 1969). It has been suggested that these effects are related in part to a decrease in water content of the reaction buffer as well as a competitive relation between these solutes and Mg^{++} or Mg^{++}-ATP involved in ATPase reactions.

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