ORGANIC NITRATE METABOLISM[1,2]

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OVERVIEW

The biotransformation of organic nitrates is initiated by a redox reaction and is manifest by the conversion of potent lipid-soluble vasodilator compounds into water-soluble metabolites which have much lower biological potency and are readily excreted in the urine. Studies by numerous workers have indicated that destruction of organic nitrates is rapidly and specifically catalyzed by hepatic glutathione-organic nitrate reductase. Blood clearance data explicitly demonstrate that the parent nitrate ester (following intravenous administration) has a very transient lifetime, whereas the nitrate metabolites circulate for hours. The duration of the vasodilator effectiveness of organic nitrates is in direct temporal correlation with blood levels of the intact ester and is completely out of phase with circulating metabolites. Thus, the parent compound appears to be the active species. On the other hand, experiments in rats, dogs, and humans indicate that after oral administration of various organic nitrates, essentially none of the parent compound is present in the circulation. A growing number of clinical studies indicate that many orally administered organic nitrates are ineffective in the treatment of angina pectoris, which is consistent with the metabolic data.

ISOLATION AND CHARACTERIZATION OF ORGANIC NITRATE REDUCTASE

The investigation of organic nitrate biotransformation began with the observation that inorganic nitrite appeared after incubation of GTN and other organic nitrates

[1]The following abbreviations are used in this review: BTTN, butanetrioltrinitrate; EGDN, ethylene glycol dinitrate; EGMN, ethylene glycol mononitrate; ETN, erythrityl tetranitrate; GDN, glyceryl dinitrate; GLUC, glucuronide; GMN, glyceryl mononitrate; GTN, glyceryl trinitrate; IS, isosorbide; ISD, isosorbide dinitrate; 2-ISMN, isosorbide-2-exomononitrate; 5-ISMN, isosorbide-5-endomononitrate; MHN, mannitol hexanitrate; ONR, organic nitrate reductase; PE, pentaerythritol; PEDN, pentaerythrityl dinitrate; PEMN, pentaerythrityl mononitrate; PETN, pentaerythrityl tetranitrate; PE-tri-N, pentaerythrityl trinitrate.

[2]Additional reviews on some aspects of this topic have previously appeared (1–3).

with blood (4–7). Inorganic nitrite formation was also observed when nitrate esters were incubated with rabbit liver homogenates (8). Furthermore, GTN administered intravenously was demonstrated to disappear rapidly from the circulation with the concurrent appearance of inorganic nitrite (5).

GTN and ETN reacted nonenzymatically with GSH to form inorganic nitrite and oxidized glutathione (9). The reduction of GTN (and ETN) by GSH was catalyzed by a hog liver enzyme in which 2 μmol of GSH were oxidized for every micromole of nitrite ion formed (9). The stoichiometry of the reaction was demonstrated by coupling the GTN transformation reaction to glutathione reductase, which catalyzed the reduction of oxidized glutathione by $NADPH_2$ (10). The coupled reaction proceeded as follows:

$$GTN + 2\ GSH \longrightarrow GSSG + NO^-_2 + \text{other products}$$

$$NADPH_2 + GSSG \xrightarrow[\text{reductase}]{\text{glutathione}} 2\ GSH + NADP$$

with the $NADPH_2$ consumed equivalent to the nitrite formed (10).

Continuous perfusion of isolated rat livers with GTN resulted in a concentration-dependent depletion of hepatic GSH and ATP (10–12). The dependence of the denitration reaction on GSH was demonstrated in an experiment in which pretreatment of rats with bromobenzene caused a 70% reduction in hepatic glutathione and resulted in a marked inhibition (90% decrease) of GTN degradation by the perfused liver (11).

The contribution of hepatic inactivation of organic nitrates was further evaluated in eviscerated rats (13, 14). GTN administered intravenously underwent rapid disappearance from the blood ($T_{1/2}$, < 1 min) of normal rats with the concurrent rapid appearance of GTN metabolites in the blood (peaked at 2–5 min). GTN disappeared from the blood of eviscerated animals much more slowly than in controls, with an apparent $T_{1/2}$ of 7–8 min. Furthermore, there was no increase in GTN metabolites in the blood with time (13). Similarly, rabbits that were functionally hepatectomized had prolonged elevated blood level of nitrates after GTN administration (14). These results were consistent with the notion that the liver was the primary site for the degradation of GTN.

The primary subcellular site of the hepatic GTN-metabolizing enzyme was in the 100,000 X g soluble fraction (15, 16). The enzyme preparation purified 100-fold from hog liver acetone powder was inhibited by cupric sulfate, stimulated by cyanide, and unaffected by dialysis or anoxia, and its optimal substrate concentrations were 5×10^{-3} M GSH and 3×10^{-3} M GTN (9). Fresh liver extracts were partially purified and yielded two distinct peaks of enzymatic activity (14,000 and 43,700 mol wt) with different relative activities toward organic nitrates (17, 18). The K_m for GSH was 1.5×10^{-5} M with GTN, 5×10^{-5} M with ETN, and 3.7×10^{-5} M with MHN (17).

Linear-chain polynitrate esters (e.g. MHN, ETN, and GTN) were rapidly transformed by rat liver 100,000 X g supernatant in the presence of GSH whereas branched-chain alcohol nitrates (e.g. PETN, PE-tri-N, and trimethylol ethane trinitrate) and anhydrides (e.g. ISD) were only slowly transformed (10). Replacement

of the nitrate by hydrogen (ETN vs 1,2,4-butanetriol trinitrate; or BTTN vs 1,4-butanediol dinitrate) or a hydroxyl (GTN > GDN >> GMN) markedly decreased the velocity of enzymatic degradation (10).

The in vitro destruction of GTN by rat liver homogenates (15, 19) or perfused rat livers (11) was markedly increased in tissue removed from animals chronically treated with phenobarbital. Similarly, the rate of disappearance of intact GTN and the appearance and excretion of metabolites of GTN were accelerated in phenobarbital-treated rats (20, 21), rabbits (22), and humans (23). The enhanced rate of nitrate degradation was apparently the result of induction of organic nitrate reductase, for inhibition of protein synthesis by DL-ethionine or inhibition of RNA synthesis by actinomycin D completely inhibited the enhancement of organic nitrate metabolism by phenobarbital pretreatment (24). On the other hand, SKF-525A pretreatment has been reported to inhibit organic nitrate metabolism in dogs and rabbits without affecting the vascular changes (25) while others could not demonstrate inhibition of nitrate metabolism whether the SKF-525A was administered in vivo or added directly to rat liver homogenates (19, 20).

The denitration of GTN by rat blood serum was independent of GSH and proceeded at a slow rate with a half-time of about 20 min (14, 18, 21, 26–28). The pH optimum was 7.8 with maximum activity at 50–57° (26, 28). The slow rate of organic nitrate degradation in vitro coupled with the apparent lack of denitration in eviscerated rats (13, 20) indicates that blood plays only a minor role in the disappearance of GTN and other organic nitrates from the circulation. The exception was MHN, which was rapidly metabolized in blood (27).

ISOLATION AND CHARACTERIZATION OF ORGANIC NITRATE METABOLITES

Metabolic Pathway in the Degradation of Glyceryl Trinitrate

Incubation of GTN with liver homogenates from several species led to inorganic nitrite formation (8, 9, 15, 16, 29). The transformation of GTN in the presence of GSH produced two organic nitrate products which were less lipid soluble than the parent molecule (15). Chromatographically, there were no differences in the GTN metabolites produced by (*a*) the nonenzymatic reaction between GTN and GSH, (*b*) the liver enzyme–catalyzed reaction of GTN and GSH, (*c*) the urinary excretion products following GTN administration to adult rats, and (*d*) synthetic 1,3- and 1,2-glyceryl dinitrate (10, 15). The time course for degradation of GTN in the rat liver homogenate was similar to the rate of the formation of 1,3- and 1,2-GDN (10). Traces of glyceryl mononitrate but no glycerol were found. The total of the nitrate groups found in GDN and inorganic nitrite accounted for 90% of the nitrate groups in the GTN that had undergone enzymatic reaction (10). Recirculation of GTN through an isolated perfused rat liver resulted in an extremely rapid disappearance ($T_{½}$, 1 min) of the parent compound consistent with its V_{max} for GSH-organic nitrate reductase. The total of the GDN, GMN, and inorganic nitrite that rapidly appeared during the liver perfusion accounted for all the GTN metabolized (11). In summary, the major route for GTN transformation appeared to be denitration

in the presence of GSH. One molecule of GTN reacted with two GSH to release one inorganic nitrite ion from either the 2- or 3-position to form 1,3- or 1,2-GDN. The denitration of GDN proceeded at only 2–5% of the rate for GTN (10). GMN was practically unaltered by the liver enzyme; this was in agreement with the finding that no glycerol was found after GTN degradation (9, 10, 20, 29).

Administration of ^{14}C-GTN to intact rats resulted in the appearance of ^{14}C carbon dioxide in the exhaled air, which reflected the total in vivo degradation of the parent molecule (20, 30). Pretreatment of rats with phenobarbital of SKF-525A had no effect on GTN oxidation, nor was there an enhancement of CO_2 released in GTN-tolerant animals (11). Eviscerated rats with or without nephrectomy were unable to oxidize GTN to CO_2. Because eviscerated rats readily oxidized glycerol to CO_2, the interruption in the GTN degradative pathway must lie somewhere in the denitration sequence (20). No CO_2 production was detected following incubation of GTN with homogenates of brain, heart, intestine, kidney, liver, lung, skeletal muscle, spleen, stomach, and blood (20). On the other hand, kidney, brain, liver, and muscle were capable of oxidizing glycerol to CO_2 (20). Thus, if GTN would have been degraded to glycerol it would have been converted to readily detectable CO_2. Furthermore, when GTN was incubated with GSH in the presence of liver homogenates, no glycerol could be detected (9, 20). Thus, there did not appear to be active enzyme system in liver (or the other tissues tested) capable of catalyzing the complete denitration of GTN metabolites of glycerol.

The GTN degradation and elimination sequence is illustrated in Figure 1. Intravenous administration of GTN to rats was associated with an extremely transient blood level of the unchanged parent compound [$T_{1/2}$ of 1 min (27)]. The GTN was apparently rapidly degraded by GSH-ONR to 1,3- and 1,2-GDN (10, 15). The disappearance of GTN from the blood proceeded at the same rate as rose bengal (^{131}I-labeled), which is a marker of hepatic function and is normally cleared from the plasma in one transit across the liver (27). GDN in turn was degraded to GMN by the same liver enzyme but at a much slower rate than the intact nitrate ester (10). The blood metabolite level consisted of 1,3-GDN and GMN. The half-time to reach the peak blood level of the nitrate metabolites was about 30 sec for GTN. The rate of disappearance of organic nitrate metabolites from the blood, after intravenous administration to rats, was slower than the appearance rate by several orders of magnitude (27). Metabolites of GTN disappeared at a first-order rate, with a clearance $T_{1/2}$ of 4 hr. The blood disappearance of the organic nitrate was regulated by the rate at which the metabolites were excreted by the kidney into the urine (31). The urinary metabolites detected after treatment of rats with GTN were identified as GMN, 1,3-GDN, and 1,2-GDN (15, 20, 21, 30). The major urinary metabolite was GMN, and the total GMN and water-soluble metabolites (presumably including glycerol) made up 80% of the excreted label (20). After intravenous administration to rabbits of 1,3-GDN, 1,3-GDN and GMN were found in the urine, whereas after intravenous administration of 1,2-GDN only GMN was detected (21). Thus, the enzymatic denitration of glyceryl trinitrate and other organic nitrates led to the generation of water-soluble metabolites that were readily elminated in the urine. A portion of the labeled compound was converted to CO_2, which was then exhaled;

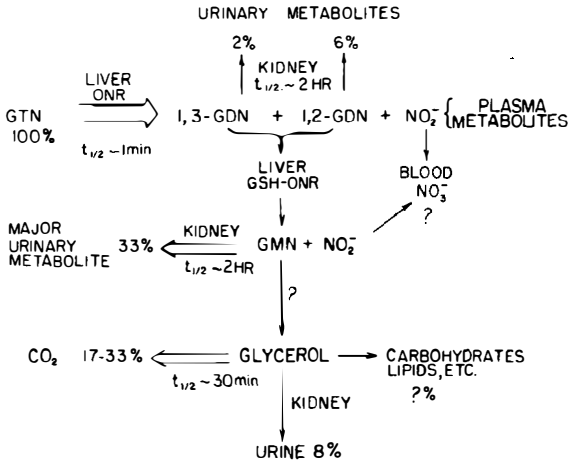

Figure 1 Schematic representation of the in vivo metabolism, clearance, and excretion of glyceryl nitrates following the administration of ^{14}C-GTN to rats. The percentage indicates the fraction of the original ^{14}C-radioactivity administered as GTN. The urinary metabolites were from pooled 24-hr samples (20). The exhaled CO_2 data represent the total for 24 hr (20, 30). The blood disappearance and urine appearance data were taken from references (27, 31). All the half-times ($T_{1/2}$) were from in vivo experiments.

thus less than 100% of the original label appeared in the urine (20, 30). Finally, the fate of the formed inorganic nitrite has not been extensively studied. Rat liver and kidney homogenates have been demonstrated to catalyze the oxidation of inorganic nitrite to inorganic nitrate (32).

GTN administered sublingually to humans produced peak plasma levels of the parent compound in the first blood sample at 4 min, and the rate at which at least half of the intact GTN was cleared from the blood was 1–3 min (33). A transient tachycardia was associated with the transient duration of circulating GTN.

Metabolic Pathway in the Degradation of Isosorbide Dinitrate

The plasma levels following intravenous administration of unchanged ISD to dogs, rats, and rabbits peaked at 2 min and then disappeared rapidly with a half-time of about 5 min (27, 34, 35) (Figure 2). The initial biotransformation occurred by denitration in the presence of hepatic GSH-organic nitrate reductase (10, 27). The blood clearance rate of the ISD metabolites was very slow ($T_{1/2}$ of 2.5 hr) (17, 34). The major plasma metabolite was 5-isosorbide mononitrate (5-ISMN-isosorbide-5-endomononitrate) (Figure 2). The plasma clearance of 2-isosorbide mononitrate (isosorbide-2-exomononitrate) paralleled that of the 5-isomer but at only approximately one seventh the concentration, thereby indicating a stereospecificity in the biotransformation (34). Low levels of isosorbide also appeared in the plasma. The primary metabolite in the plasma was 5-ISMN (65% of the administered ISD) which had only one thirtieth the coronary-dilating activity of the parent compound

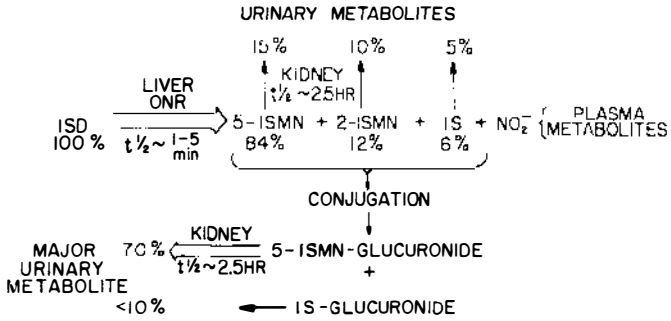

Figure 2 Metabolic pathway in the degradation of ISD. After intravenous administration, ISD is rapidly cleared from the blood (27, 34, 35) following denitration by organic nitrate reductase (10, 27) with the appearance of 5-ISMN, 2-ISMN, and free IS. The relative ratio of ISD metabolites in the blood is 14:2:1 for 5-ISMN:2-ISMN:IS (34); thus about 65% of the administered drug in the plasma is 5-ISMN, which has only one thirtieth to one hundredth the coronary-dilating activity of the parent compound (63, 64) but circulates 20–50 times longer than the ISD. The slow clearance from plasma (27, 34) is the result of renal filtration and conjugate formation. In 24 hr, 80–100% of the administered dose appears in the urine (33). Essentially no parent ISD was detected in the plasma after oral administration (34). The percentages indicated the portion of the total radioactivity originally administered (34, 38, 39). GSH-ONR is the abbreviation for GSH-organic nitrate reductase.

but circulated 20-50 times longer than the ISD (34). Unlike GTN, the disappearance of the metabolites of ISD from the blood was not only determined by renal filtration but also required conjugation leading to the formation of glucuronides of 5-ISMN and of IS (36). A quantitatively different blood clearance pattern was observed following oral administration of ISD to dogs (34). Only trace amounts of the unchanged material appeared in the circulation. The major metabolite 5-ISMN reached its peak concentration at a later time (30 min); 2-ISMN was again found to parallel 5-ISMN but at a much lower concentration. Five human volunteers treated sublingually with ISD had peak blood levels of intact ISD by 6 min, and the blood clearance rate of the parent compound was rapid, with 60% disappearance occurring by 15 min or less (37). An oral dose of ^{14}C-ISD was rapidly absorbed and 99% eliminated principally as the isosorbide glucuronide in the urine by human subjects (38).

The rate of appearance of urinary metabolites of ISD reflected the rate of blood clearance of the metabolites (Figure 2). The accumulated sum of the ISD urinary metabolites approached 100% of the administered radioactive ISD at 24 hr (31). A similar pattern was observed in dogs treated orally or intravenously with ISD (34). No unchanged ISD was found in the urine. Twenty to thirty percent of the carbon skeleton of ISD was excreted as neutral metabolites, principally IS, 5-ISMN, 2-ISMN, and isoidide (34, 36, 39). The remainder primarily consisted of the ether glucuronide of 5-ISMN and ether monoglucuronides of IS.

Rats orally treated with the dinitrates of isosorbide and its stereoisomers isomannide and isoidide had only trace amounts of the parent compounds in the urine (40). The mononitrates, the denitrated alcohols, and large amounts of conjugated mononitrates were qualitatively identified. Evidence for inversion from the endo- to the exo-position was provided by the finding that following ISD administration, isoiodide mononitrate and isoidide were also in the urine. Similarly, following isomannide dinitrate treatment, 5-isosorbide mononitrate and isosorbide were found.

Metabolic Pathway in the Degradation of Pentaerythrityl Nitrates

Incubation of PETN with rat liver homogenate and GSH resulted in a slow rate of formation of three metabolites (10) that were identified as the tri-, di-, and mononitrates of PE (28, 41, 42) (Figure 3). PE and its mono- (42%) and di- (33%) nitrate accounted for the major radioactivity in the blood but PETN and PE-tri-N were not detected in blood at any time interval studied following oral administration of PETN to mice and rats (42–45). The first urine collection (at 2 hr) following oral administration of PETN to rats contained PEMN (52% of administered dose), PEDN (33%), and PE (16%) (44). By 18 hr only PEMN (27%) and PE (73%) were present. There was no detectable PETN or PE-tri-N in the urine. Fifteen human volunteers were treated with ^{14}C-PETN and the pattern of metabolites in blood or urine of humans was the same as in experimental animals (46). PETN and PE-tri-N were not detected in blood. The major circulating metabolite was PE with lower levels of PEMN and trace amounts of PEDN (46–49). PE and PEMN were the major urinary metabolites. There was no qualitative or quantitative difference in the

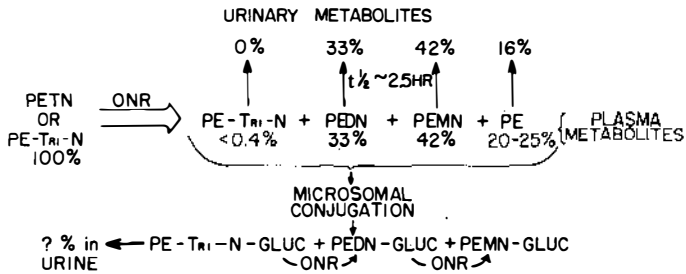

Figure 3 Metabolic pathway in the degradation of PETN or PE-trinitrate. The following abbreviations are used: PE-trinitrate (PE-tri-N), PE-dinitrate (PEDN), PE-mononitrate (PEMN), glucuronide (GLUC), and GSH-organic nitrate reductase (ONR). Two hours after oral PETN administration to rats there was no parent compound or PE-tri-N in the blood, and the major plasma metabolites were PEDN, PEMN, and PE. The percentages indicate the portion of the originally administered dose (42, 45, 49, 51, 55). The clearance of PE-tri-N metabolites from blood was 2–3 hr (43). The PE-nitrates are converted to glucuronides by liver microsomes (45, 52–54); then these PE-nitrate-glucuronides undergo further denitration by ONR (49, 53). The urinary metabolites are those found 2 hr after oral administration, and the percentage represents the fraction of the totally excreted radioactivity in the urine at 2 hr (44).

excretion patterns and blood levels exhibited by coronary artery disease patients and controls (Figure 3). PEDN, PEMN, and PE have little or no peripheral or coronary vasodilator activity and cannot account for the biological activity of the parent compound (50).

In a very recent paper intact PETN and PE-trinitrate were claimed to be present in plasma and to disappear slowly ($T_{1/2}$, 2 and 3 hr respectively) following oral administration of ^{14}C-PETN to rats (45). Their own data indicate that only 0.0006% and 0.008% of the circulating drug exist as PETN and PE-trinitrate. Furthermore, in numerous previous papers by these authors (using the same conditions as in the above report) they have concluded that "PETN and PE-trinitrate were not detected in blood at any time interval studied" in rats (44, 51), dogs (55), and humans (47, 49).

The lack of aqueous solubility of PETN has generated interest in studying the properties of the more soluble PE-tri-N. PE-tri-N administered orally was found to be absorbed faster than PETN (48, 51). PE-tri-N was partially metabolized by hepatic glutathione organic nitrate reductase (10) and by perfused rat livers (11). PE tri-, di-, and mononitrates are converted to glucuronides by liver microsomes (45, 52), and these nitrates and their glucuronides undergo further denitration by organic nitrate reductase (52–54). Following oral administration of PE-trinitrate to various species, there was little or no unchanged parent compound present in the circulation (51, 55). Even at the earliest sampling time (1 hr), there was only a trace amount of the PE-dinitrate (4%), some PE-mononitrate (28%), and predominantly PE (67%). The primary urinary metabolites following the oral administration of PE trinitrate were: PE > PE-mononitrate > PE-dinitrate (51). Biliary excretion of PE-trinitrate metabolites has been demonstrated (52). The contribution of the enterohepatic circulation was indicated by the observation that bile collection caused a 60% reduction in the radioactivity that appeared in the urine of PE-trinitrate-treated rats (52). ^{14}C PE-trinitrate was administered to humans by either the oral or sublingual route (48). No intact PE-trinitrate appeared in the blood, PE-dinitrate was present only briefly, and PE was the major circulating form of the drug.

Degradation of Other Organic Nitrate Esters

Mannitol hexanitrate was rapidly degraded by hepatic glutathione-organic nitrate reductase about seven times faster than GTN (10). The in vivo clearance of MHN from the blood of rats was extremely rapid ($T_{1/2}$, 10 sec) (27). In addition, a significant rate of blood metabolism of MHN ($T_{1/2}$, 1–2 min) further accelerated the blood clearance rate. Metabolites of MHN disappeared very slowly from rat blood after intravenous administration; the $T_{1/2}$ for clearance from the blood into the urine was 2 hr (27).

Erythrityl tetranitrate was metabolized three times faster than GTN by liver homogenates or by isolated perfused rat livers (10, 27), and the total amount of organic nitrate metabolized in the perfusion correlated closely to the previously measured V_{max} with GSH-organic nitrate reductase (10).

Ethylene glycol dinitrate subcutaneously injected in rats produced peak blood levels of the parent compound in 30 min and fell to zero by 8 hr; the metabolites

ethylene glycol mononitrate, inorganic nitrite, and inorganic nitrate were rapidly produced reaching a peak at about 1–3 hr and falling to zero in 12 hr (56). Mostly inorganic nitrate was present in the urine; there was no EGDN, a trace of EGMN, no inorganic nitrite.

Propylene glycol 1,2-dinitrate was degraded in vitro (rat blood) or in vivo (rats, s.c.) into 1- and 2-isomers of propylene glycol mononitrate, inorganic nitrite, and inorganic nitrate (57). The mononitrates are further metabolized in vivo with the result that only small amounts were excreted and the major urinary metabolite was inorganic nitrate.

1,-Chloro-2,3-propanediol dinitrate was metabolized in dogs (orally administered) to 1-chloropropyl-3-glucuronide-2-nitrate and 1-chloropropyl-2-glucuronide-3-nitrate (58). Presumably the initial degradation was hepatic denitration and subsequent formation of the glucuronide prior to urinary excretion.

RELATIONSHIP BETWEEN ORGANIC NITRATE METABOLISM AND BIOLOGICAL ACTIVITY

Temporal Relationship Between Plasma Levels and Biological Activity

Administration of organic nitrates into jugular veins of anesthetized rats resulted in a transient vasodepression, usually of a 1- to 4-min duration, depending on the dose (31). There was essentially no difference in the duration of the fall in blood pressure produced by intravenous injection of GTN or by the so-called longer-acting nitrates, MHN, PETN, ISD, or ETN. On the other hand, injection of the above nitrates into the portal vein produced no blood pressure fall, suggesting that the liver has the capacity in a single passage to prevent organic nitrate-induced vasodepression by destroying the intact vasoactive ester. The only exception found was 1,2,4-butanetriol trinitrate, which in high doses exceeded the liver capacity and produced a vasodepression (31). This compound was previously found to be more slowly degraded by glutathione-organic nitrate reductase than the other drugs tested (10). In different investigations in rats, GTN and PE-trinitrate were 50 to 100 times more potent as a vasodepressor when intravenously administered than when administered through the portal vein (59). Extremely high doses of GTN or PE-trinitrate given by intraduodenal or oral administration were reported to cause a vasodepression, but there was only a very transient (1–2 min) decrease in coronary vessel resistance with the PE-trinitrate. However, there was no evidence of blood levels of intact parent compound (i.e. either PETN or PE-trinitrate) nor substantial amounts of the initial metabolite (PE-dinitrate) following oral administration of either PE-tetranitrate or PE-trinitrate (47, 48). The oral administration of very high doses of organic nitrates to overcome hepatic inactivation would probably exaggerate side effects and induce tolerance to the administered agent and cross-tolerance to GTN (60).

The clearance of intact GTN, ISD, and MHN from the blood proceeded very rapidly ($T_{1/2} < 1$ min) following intravenous injection in rats (31). The blood was cleared of more than 80% of the parent nitrate in 1 min. The rates of disappearance of the metabolites were slower than their appearance by several orders of magnitude.

The approximate half-times for metabolite clearance from the blood were 2 hr for MHN, 2.5 hr for ISD, and almost 4 hr for GTN (31). Following injection of radioactive organic nitrates directly into the portal vein, little of the intact parent compound passed through the liver as an intact molecule, but there was an immediate appearance of metabolites in the circulation; however, there was no systemic vasodepression (31). The blood clearance data following intravenous administration of organic nitrates indicate that the transient duration (1–4 min) of the biological response (vasodepression) correlated directly with the transient circulation time (80% removed in 1 min) for the parent compound. The vasodepression (1–4 min) was completely out of phase with the circulation time for the nitrate metabolites ($T_{1/2}$, about 2 hr) (31).

Biological Activity of Metabolites

The comparisons of the vasodilator potency of intact organic nitrates on blood pressure or vascular resistance indicated that the denitrated metabolites were less potent than the parent compounds (49, 61–64).

GLYCERYL TRINITRATE Organic nitrates were compared as blood pressure depressants in dogs and guinea pigs (61, 62). GTN was at least 10 times more potent than glyceryl dinitrate and 40 times more potent than inorganic nitrite, and GMN and inorganic nitrate were inactive (61). Compared with GTN, GDN was only 2% as active in lowering guinea pig blood pressure, 0.2% as active in relaxing rabbit aorta strips, and 5% as active in decreasing dog hindleg resistance (62). PETN and butanetriol trinitrate were also more potent than their denitrated metabolites (62). In general, the more active vasodepressor compounds were the nitrates with high oil/water partition coefficients. The less lipid-soluble compounds (i.e. denitration replaces lipid-soluble nitrate ester group with a water-soluble hydroxyl group) required progressively higher doses to elicit an equivalent fall in blood pressure (61).

ISOSORBIDE DINITRATE The order of the coronary vasodilator potency (i.e. the dose required to produce a 10 ml/min change in blood flow in the circumflex coronary artery in dogs) was as follows: GTN, 1 μg; ISD, 100 μg; 2-ISMN, 300 μg; and 5-ISMN, 3000 μg (63). The order of potency required to reduce pressure by 10 mm Hg in the perfused dog hindlimb was GTN, 0.1 μg; ISD, 2 μg; 2-ISMN, 10 μg; and 5-ISMN, 200 μg (63, 64). 5-ISMN was demonstrated to be the predominant circulating metabolite in blood after either oral or intravenous administration of ISD (34). Much lower concentrations of the 2-ISMN metabolite were present. 5-ISMN was only one thirtieth to one hundredth as potent as ISD (63, 64). These observations strongly supported the evidence that the intact parent nitrate ester was the biologically active species and that denitration inactivated the compound.

PENTAERYTHRITOL TRINITRATE An explanation to justify the use of PETN or PE-trinitrate as long-acting compounds was the demonstrated presence of persisting levels of PE-dinitrate or mononitrate metabolites in blood (42, 43, 45, 49, 51, 55). However, these metabolites proved to be without significant vasodilator activity in

comparison to the parent compound; "therefore their persistent plasma levels cannot be responsible for the sustained action of PE-trinitrate in man" (65). PE-trinitrate was one fifth as potent as GTN as a coronary vasodilator and as a systemic vasodepressor. PE-dinitrate was only one fiftieth as potent as the PE-trinitrate, PE-mononitrate was one hundredth the potency of PE-trinitrate, and PE was completely inactive (50, 65). Furthermore, cross tolerance occurred between the active nitrate, PE-trinitrate, and its nearly inactive metabolites (65). Thus, accumulation of inactive metabolites in the plasma following chronic drug treatment might be expected to diminish response to an active agent needed acutely (e.g. GTN during angina).

CONCLUSIONS

The qualitative and quantitative pattern of biotransformation of organic nitrates in humans was the same as in experimental animals. The primary route of degradation of organic nitrates was enzymatic denitration by hepatic GSH-organic nitrate reductase. Fresh human liver biopsy samples had the same maximum enzyme velocity for GSH-dependent denitration as did rat liver, which was the model species for many of the nitrate biotransformation studies (31). Since these comparisons were on a liver weight basis, humans actually had at least 200 times more enzyme available than rats.

The principal finding of nitrate metabolism experiments in rats, dogs, and humans was that following oral administration of various organic nitrates, little if any of the parent compound was present in the circulation to relax vascular smooth muscle. Thus, following oral administration, the nitrates were absorbed into the portal circulation and were rapidly and completely degraded by the liver (GSH-organic nitrate reductase) before reaching the systemic circulation; therefore, they could have little chance of producing vasodilation.

Consistent with these observations are a large number of clinical studies that indicate that orally administered organic nitrates are ineffective. According to Modell (66), "The history of failure with so-called long-acting nitrates is an unbroken one." One would anticipate that long-term effects of a "long-lasting" vasodilator would be associated with sustained blood levels of the active compound. This has not proven to be the case. After oral administration or shortly after intravenous administration of intact nitrates, the predominant circulating species have been metabolites. The primary circulating metabolites of GTN, PETN, PE-trinitrate, or ISD are much too low in potency to account for the biological effect. The only other metabolite thus far tested was mannitol pentanitrate (67), which has very weak vasodilator activity in dogs. The circulating metabolites although unassociated with the time course of the nitrate-induced vasodilation would still be involved in provoking side effects. Severe headaches, which were out of time phase with biological effects and not accompanied by any change in blood pressure or heart rate, were noted following GTN treatment of human volunteers (23).

The sublingual administration of organic nitrates still represents a rational approach to the acute relief of an angina pectoris attack. ETN, MHN, and triethanola-

mine trinitrate were effective when administered sublingually instead of orally (68). Buccal adsorption of drug initially avoids hepatic destruction, and since only about 15% of the cardiac output is delivered to the liver, a transient but effective circulating level of intact organic nitrate would occur prior to inactivation. Little difference in the duration of action of various organic nitrates taken by the sublingual route should be anticipated because the hepatic organic nitrate reductase is highly active in humans (31). Such a comparison was carried out by adjusting the sublingual dosage of ISD and GTN to be equipotent regarding circulatory changes in triple product and in exercise tolerance in patients (69). Under these conditions there was no significant difference in the duration of action of these nitrates in the patients tested. Hepatic destruction of organic nitrates following sublingual administration should be dependent only on the rate at which they are delivered to the liver. It is conceivable that different patients could vary in their time course of response to sublingual nitrates. The amount of drug delivered to the liver could be influenced by such conditions as differential volumes of distribution for the various organic nitrates or radically altered (by stress, exercise, or angina) hepatic blood flow.

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