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Metabolism of and vascular responses to glyceryl trinitrate in the eviscerated rat

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AN ENZYME isolated from the soluble fraction of liver homogenates (glutathione-organic nitrate reductase) catalyzes the denitration of glyceryl trinitrate (GTN).[1,2] GTN metabolism in isolated, perfused rat livers proceeds extremely rapidly, and is primarily dependent on glutathione-organic nitrate reductase activity.[3]

It is possible that blood or organs other than the liver enzymatically degrade a significant portion of administered GTN. It, therefore, becomes critical to evaluate the role of the liver in organic nitrate degradation since many "long-acting" organic nitrates are taken orally by patients as prophylactic treatment in angina pectoris. Presumably, drugs reabsorbed from the gastrointestinal tract go directly to the liver via the portal circulation.

In order to further clarify the postulated hepatic detoxication of organic nitrates, totally eviscerated rats were studied. The metabolism and duration of vascular responses in these preparations constitute the subjects of this report.

Eviscerations were performed as described by Markowitz *et al.*[4] and Farris and Griffith.[5] Preparations were judged to be acceptable when blood pressure and heart rate were both at or near control values. In all, there was less than a 10 per cent fall in mean blood pressure and the animals had a mean survival time of 8 hr when supplemented with 200 mg of glucose/kg of body weight/hr.

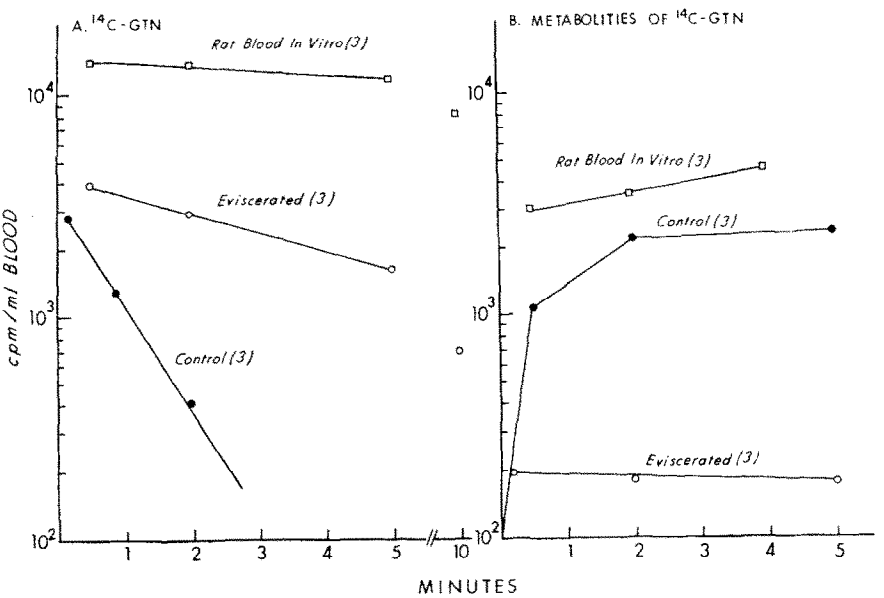

FIG. 1(A). Time course of disappearance of ^{14}C-GTN from the blood. GTN-1,3-^{14}C (0·2 μc in 0·2 mg of unlabeled carrier GTN) was administered through a jugular vein cannula to a heparinized, pentobarbital-anesthetized rat. Carotid blood samples (0·3 ml) were withdrawn at the appropriate time and immediately injected into 5 ml of petroleum ether and rapidly mixed. Petroleum ether (2 × 5 ml) quantitatively extracted all the parent GTN leaving the metabolites in the blood (which agrees with the quantitative recovery of GTN from a mixture of glyceryl nitrates as described by Dunstan *et al.*[7]). The pooled extract was evaporated and counted on a liquid-scintillation counter. (B) Time course of appearance of GTN metabolites in the blood. The GTN free blood (after 2 × 5 ml petroleum ether extraction in Fig. 1, A) was extracted with 5 ml of absolute ethanol (which quantitatively removes the GTN metabolites), evaporated, and counted. Separation of parent GTN and its metabolites was confirmed by thin-layer chromatography.

GTN-1,3-^{14}C was synthesized from glycerol-1,3-^{14}C (New England Nuclear) by the nitration procedure of Lawrie[6] and purification was accomplished according to the method described by Dunstan *et al.*[7] The final product was chromatographically and radiochemically pure and the alcoholic stock solution had a specific activity of 1 μc/μmole.

After intravenous administration of ^{14}C-GTN in control animals, there is a rapid disappearance of this compound from the blood. The biological half-time is less than 1 min (Fig. 1, A). There was a simultaneous rapid appearance of GTN metabolites in the blood (Fig. 1, B), which reached peak concentrations at 2–5 min and disappeared with $t_{\frac{1}{2}}$ of 3–4 hr (not shown).

The time course of disappearance of ^{14}C-GTN from the blood of eviscerated animals proceeds much more slowly than in controls. The apparent half-time is 7–8 min. Furthermore, there is no increase in the initial low concentrations of GTN metabolites in the blood with time (Fig. 1). These data establish that the degradation *in vivo* of GTN is carried out primarily in the liver and presumably results from the reaction of glutathione-organic nitrate reductase.[1–3]

The slow rate of degradation of GTN in rat blood *in vitro* has been reported by Di Carlo and Melgar.[8] They state that denitration of GTN by rat blood serum has a half-time of about 20 min. This slow rate of denitration *in vitro* coupled with the apparent lack of degradation in eviscerated rats would indicate that blood plays only a small role in the disappearance rates of GTN from the circulation of intact animals.

The above results are consistent with the notion that the liver is essential for the degradation *in vivo* of GTN.

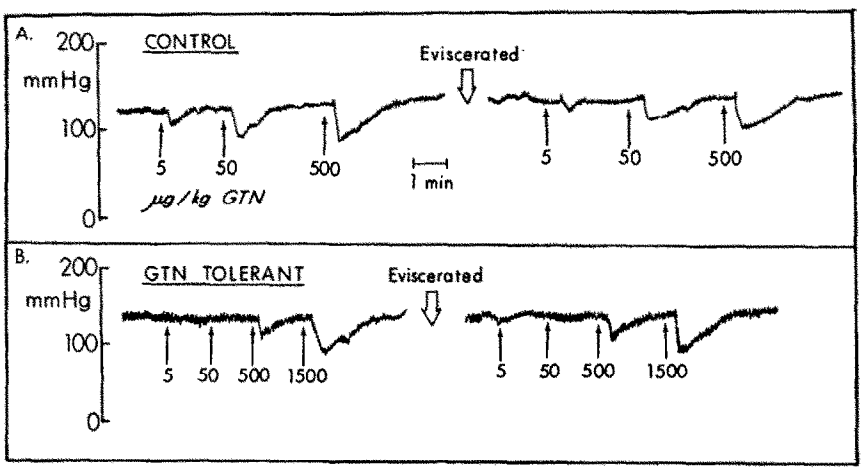

FIG. 2. Blood pressure response of control (N = 6) and tolerant (N = 3) rats to GTN before and after evisceration. The elapsed time for the evisceration was about 30 min. GTN tolerance was produced by treating rats with GTN—100 mg/kg (s.c.), 3 times daily for 3 days.[11]

It is likely that the fall of blood GTN levels in the eviscerated rat represents tissue distribution of this lipid soluble compound coupled with slow denitration by the blood.

The administration of GTN (i.v.) to pentobarbital-anesthetized rats causes a dose-dependent fall in blood pressure (Fig. 2). The duration of the vasodepression is 1–2 min which compares favorably with the disappearance of parent GTN from the blood ($t_{\frac{1}{2}}$ = 1 min). The time course of the disappearance of the GTN metabolites from the blood ($t_{\frac{1}{2}}$ = 3–4 hr) is completely out of phase with the biological response. The GTN metabolites have been previously shown to have low vasodilator activity.[1,9] These observations of correlation of biological activity with GTN blood levels differ markedly from the report of Bogaert *et al.*[10] These workers administered 1 mg/kg of unlabeled GTN to rabbits and dogs and found that the transient blood pressure response did not parallel the plasma organic nitrate levels which reached a maximum at 10–20 min after injection. The most likely interpretation of their results is that they were not distinguishing between the plasma levels of the parent compound and its less active metabolites.

Since the evidence for the lack of GTN metabolism in the absence of the liver is quite clear, it was anticipated that the duration of depressor action of GTN would be greatly prolonged in the liverless animal. Surprisingly, however, there was no change in either the magnitude or duration of action of GTN in eviscerated rats as compared with controls (Fig. 2). Bilateral nephrectomy in the eviscerated or control animals makes no difference in the magnitude or duration of vascular action of GTN (data not shown).

If metabolism is the limiting factor in GTN vasodepression, then the administration of 500 μg/kg to control rats would be expected to maintain a blood level above 5 μg/kg (minimal effective vasodilator dose) for 7 min [7 $\times$ 1 min ($t_{\frac{1}{2}}$)]. Comparably, 500 μg/kg of GTN in the eviscerated rat would last almost 1 hr. The duration of the vascular response of GTN is obviously independent of hepatic metabolic alterations of the compound.

It is unlikely that the similarity of the time course and magnitude of blood pressure depression in control and eviscerated animals is determined by cardiovascular reflexes. In neither preparation are there discernible changes in heart rate. The peripheral beds capable of vasoconstriction are so different in the preparations as to make it virtually impossible to have equal responses to equal dose levels of drug.

The transient duration of the GTN vasodepression cannot be explained by urinary loss, renal conversions, drug metabolism, or distribution of GTN in some larger compartment than plasma water. Several possible explanations of this self-limiting vascular response exist. One explanation is that the lack of sensitivity of receptors in the vascular smooth muscle requires a regeneration period analogous to other excitable tissue after stimulation. This period of refractoriness (or acute tolerance) could be the time required for such processes as: membrane repolarization; replenishing an ion or chemical store; or conformational change in the receptor protein. This notion could possibly explain the development of chronic tolerance to GTN,[11] such that the sustained exposure of receptor sites to GTN eventually diminishes the ability of the cells to readjust for subsequent stimulation. Indeed, GTN tolerance was not reversed by evisceration (Fig. 2); thus, such factors as enhanced non-hepatic metabolism or binding do not appear to account for the lack of responsiveness to GTN. In addition, previous reports indicate that tolerance to the effects of GTN does not depend on hepatic biotransformation.[3,11] More likely, chronic tolerance would appear to involve an altered receptor response in vascular smooth muscle.

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