Binding and Metabolism of Nitroglycerin by Rat Blood Plasma (33889)

FREDERICK J. DICARLO AND MYRIAM D. MELGAR

Biochemistry Department, Warner-Lambert Research Institute, Morris Plains, New Jersey 07950

Although protein binding is a component of the metabolic profile of a drug, this information was lacking for nitroglycerin. Drugs which are bound firmly by blood protein are generally considered to offer possible long duration of activity. Nitroglycerin is well known to act quickly, but briefly. Accordingly, it might have been expected to show little or no affinity for plasma protein. This point was investigated to extend understanding of the metabolism of organic nitrates.

Organic nitrates are known to undergo biotransformation by blood erythrocytes (1, 2) and plasma (2). Therefore, the present study provided an opportunity to determine the binding of nitroglycerin metabolites.

Materials and Methods. (1, 3-^{14}C)-Glyceryl trinitrate was synthesized at Evans Research and Development Corp. from (1, 3-^{14}C-glycerol by the nitration procedure of Lawrie (3). Purification according to Dunstan *et al.* (4) yielded a product with 99.9% radiochemical purity. In order to minimize the danger of explosion, the product was diluted with 19 parts by weight of C. P. lactose. The specific activity of the mixture was 0.18 mCi/g.

Binding experiments. In order to limit the extent of nitroglycerin metabolism, the ultrafiltration technique of Rehberg (5) was used at 0°. The centrifugation time was 45 min. The concentration of nitroglycerin was varied from 50 to 500 μg/ml of fresh rat blood plasma. The volume employed was 2.0 ml for each experiment.

Immediately following each ultrafiltration, aliquots of the plasma were taken for counting and the balance was diluted with an equal volume of methanol to precipitate proteins and to halt enzymatic reactions. The mixtures were centrifuged and the supernatants were used for thin-layer chromatography. The filtrates were used directly for counting and thin-layer chromatography.

Radioactivity counting. Scintillation spectrometry was used to measure the ^{14}C concentrations. The scintillation solution consisted of 7.0 g of 2,5-diphenyloxazole; 0.3 g of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene; and 100 g of naphthalene in 1.0 liter of freshly distilled dioxane.

Thin-Layer Chromatography. Thin-layer chromatograms were developed on 2 $\times$ 8-in. glass plates coated to a thickness of 250 μ with silica gel G. The solvent consisted of benzene:ethyl acetate:acetic acid, 16:4:1 (v/v/v). The ascending technique with chamber saturation gave the best resolution of nitroglycerin, glyceryl-1,2-dinitrate, and glyceryl-1-1,3-dinitrate (6). The chromatograms were scanned with a Packard model 7201 radiochromatogram scanner to determine the R_f values of the radioactive bands. The areas of the peaks on the scans were measured with a planimeter to determine the relative quantity of each labeled compound present.

Results. The protein volume of plasma was taken into account in calculating the percentage of drug bound (7). Using values of 6.0% for the protein content (8) and 0.75 for the specific gravity of the protein, the water volume of the plasma was estimated $100 - (6.0 \times 0.75) = 95.5\%$.

Nitroglycerin was attacked enzymatically during the course of the binding experiments (Table I). Although the temperature was low, the period of contact between the substrate and serum was long enough to degrade from 8 to 17% of the drug. The only products formed in significant quantities were the glyceryl dinitrates. In each experiment, far more glyceryl-1,3-dinitrate than glyceryl-1,2-dinitrate was produced; the mean proportion between the isomers was 1.9.

Approximately the same proportion of the available radioactivity was bound by the rat plasma over the tenfold range of ^{14}C-nitroglycerin concentration which was inves-

TABLE I. Nitroglycerin Biotransformation during Binding Determination.

Nitroglycerin (μg/ml of plasma)	Fraction	Composition[a] (%)		
		Nitroglycerin	Glyceryl-1,3-dinitrate	Glyceryl-1,2-dinitrate
50	Plasma	84	11	5
	Filtrate	76	18	6
100	Plasma	85	9	6
	Filtrate	81	14	5
200	Plasma	82	12	6
	Filtrate	76	18	6
500	Plasma	92	5	3
	Filtrate	91	7	2

[a] The R_f values were: nitroglycerin, 0.65; glyceryl-1,3-dinitrate, 0.47; glyceryl-1,2-dinitrate, 0.35; glycerol, 0.00. Neither glyceryl-1-nitrate nor glyceryl-2-nitrate was detected; both migrate to R_f 0.10 in this solvent, but have been resolved otherwise (6).

tigated (Table II). Using the nitroglycerin degradation data presented in Table I, binding values were calculated for the glyceryl dinitrates as well as for nitroglycerin. About 60% of the nitroglycerin was found to be bound. Glyceryl-1,2-dinitrate binding was approximately the same, but the isomeric glyceryl-1,3-dinitrate was bound only to the extent of about 35%. Table II also shows the binding data in terms of the quantities of the three compounds which are bound per unit weight of protein. From this view it is clear that plasma protein has a high capacity for holding nitroglycerin, but seems to have much lower capacity for the glyceryl dinitrates. The weight of nitroglycerin bound per milligram of protein was proportionate to the concentration of the drug. This relationship also held for glyceryl-1,2-dinitrate, but the protein seemed to approach saturation with glyceryl-1,3-dinitrate.

Discussion. The observation that glyceryl-1,2-dinitrate was bound about as extensively as nitroglycerin and far more extensively than glyceryl-1,3-dinitrate suggests that the binding of nitroglycerin may be attributable mainly to its adjacent nitrate ester groups. The finding that plasma protein shows moderate affinity for nitroglycerin and strong affinity for pentaerythritol tetranitrate (2) is consistent with the relative duration of activity of these drugs. Also consistent with the clinical data is the observation that nitroglycerin is metabolized much faster than pentaerythritol tetranitrate by plasma (2). A similar relationship was found previously by Needleman and Hunter (9) who observed that a hog liver enzyme preparation rapidly de-esterfied nitroglycerin, but only slowly attacked pentaerythritol tetranitrate.

Since the 1- and 3-positions of nitroglycerin are identical, random cleavage of the three nitrato groups would yield twice as much of the 1,2-dinitrate as of the 1,3-dinitrate. This

TABLE II. Binding of Nitroglycerin and Glycerol Dinitrates by Rat Blood Plasma.

Nitroglycerin (μg/ml of plasma)	Bound (%)				Bound (μg/mg of protein)		
	^{14}C	Nitroglycerin	Glyceryl-1,3-dinitrate	Glyceryl-1,2-dinitrate	Nitroglycerin	Glyceryl-1,3-dinitrate	Glyceryl-1,2-dinitrate
50	57.8	62	31	50	424	31	25
100	60.6	62	38	67	878	55	67
200	54.4	58	32	55	1585	128	110
500	53.2	54	34	68	4140	142	170

proportion of glyceryl dinitrates was approximated in the urine of rats treated with nitroglycerin (10). However, the metabolism of nitroglycerin by plasma produced a dinitrate mixture with a 2:1 preponderance of glyceryl-1,3-dinitrate. This result indicates a 4:1 preferential enzymatic attack upon the secondary nitrate ester grouping at C-2, and is reminiscent of the selective removal of the 4-nitrato group from mannitol hexanitrate (11, 12) and of the 3-nitrato group from dulcitol hexanitrate (13) by pyridine. The fact that the observed preferential cleavage of nitroglycerin by the serum was not reflected by the composition of the urinary dinitrates implies that random de-esterification may be the main metabolic pathway in the total animal. Indeed, the major proportion of nitroglycerin degradation may well be effected by hepatic organic nitrate reductase (9). Whether or not such is the case, it is our view that the drug metabolites in the systemic circulation may have greater pharmacological significance.

Summary. The ultrafiltration technique was employed to measure the extent of ^{14}C-nitroglycerin binding by the proteins of rat blood plasma. Over the concentration range of 50–500 μg of nitroglycerin/ml of plasma, approximately 60% of the drug was bound. During the course of the binding experiments, nitroglycerin was metabolized to glyceryl dinitrates. Twice as much glyceryl-1,3-dinitrate was formed as glyceryl-1,2-dinitrate, indicating a strongly preferential attack upon the 2-position rather than upon the terminal positions. The metabolites were also bound by plasma proteins with glyceryl-1,2-dinitrate being held more extensively (about 60%) than glyceryl-1,3-dinitrate (35%).

1. Crandall, L. A., Jr., J. Pharmacol. Exptl. Therap. **48**, 129 (1933).

2. DiCarlo, F. J., Coutinho, C. B., Sklow, N. J., Haynes, L. J., and Crew, M. C., Proc. Soc. Exptl. Biol. Med. **120**, 705 (1966).

3. Lawrie, J. W., "Glycerol and the Glycols," p. 318. Chem. Catalog Co., New York (1928).

4. Dunstan, I., Griffiths, J. V., and Harvey, S. A., J. Chem. Soc. 1319, 1325 (1965).

5. Rehberg, P. B., Acta Physiol. Scand. **5**, 306 (1943).

6. Crew, M. C. and DiCarlo, F. J., J. Chromatog. **35**, 506 (1968).

7. Davis, B. D., J. Clin. Invest. **22**, 753 (1943).

8. Moore, D. H., Levin, L., and Snelser, G. K., J. Biol. Chem. **157**, 723 (1945).

9. Needleman, P. and Hunter, F. E., Jr., Mol. Pharmacol. **1**, 77 (1965).

10. DiCarlo, F. J., Crew, M. C., Haynes, L. J., Melgar, M. D., and Gala, R. L., Biochem. Pharmacol. **17**, 2179 (1968).

11. Hayward, L. D., J. Am. Chem. Soc. **73**, 1974 (1951).

12. Brown, J. R. and Hayward, L. D., Can. J. Chem. **33**, 1735 (1955).

13. McKeown, G. G. and Hayward, L. D., Can. J. Chem. **33**, 1392 (1955).

Received Sept. 3, 1968. P.S.E.B.M., 1969, Vol 131.