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COMMUNICATIONS

Metabolite inhibition of nitroglycerin metabolism in sheep tissue homogenates

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The metabolism of nitroglycerin in sheep tissue homogenates has been examined using tritiated nitroglycerin and a HPLC separation procedure. Nitroglycerin was metabolized by liver, lung, muscle, arterial and venous tissue to its dinitrometabolites and subsequently to mononitroglycerin. Addition of the dinitrometabolites substantially inhibited the degradation of nitroglycerin in all tissue homogenates.

Knowledge of organic nitrate disposition and determinants of their clinical effects remains limited. Some published data are suspect due to failure to recognize or account for sorption of organic nitrates to plastic components of infusion sets or sampling devices (Cossum et al 1978), metabolism in blood before analysis (Armstrong et al 1980) or extraction of organic nitrates across vascular beds in-vivo (Armstrong et al 1982).

Understanding the extent and nature of organic nitrate extraction across vascular beds is necessary in optimizing the clinical usage of these compounds. As only about 1% of the total amount of nitroglycerin in the body at any time is found in the plasma (McNiff et al 1981), minor changes in its tissue binding and metabolism will lead to marked alterations in plasma concentrations (Fung 1983). We have examined various tissues for their capacity to metabolize nitroglycerin. Tissues from sheep were used to provide preliminary data. As significant concentrations of the dinitrometabolites are found during nitroglycerin administration (Miyazaki et al 1982), the effect of the presence of these metabolites on the tissue metabolism of nitroglycerin was also examined.

Materials and methods

Tritiated nitroglycerin. Tritiated nitroglycerin was synthesized by incubating [1(3)-^{3}H]glycerol (2·5 Ci mmol^{-1}) (Amersham, Australia) with a fuming nitric/sulphuric acid mixture for 30 min at a temperature less than 10 °C (Cossum & Roberts 1985). The reaction mixture was then neutralized with sodium carbonate solution, washed twice with water and then applied to a reverse phase Bondapak C_{18} (30 cm × 3·9 mm i.d.) HPLC column (Waters Assoc.) using a mobile phase of 30% methanol in water (Crouthamel & Dorsch 1979). At a flow rate of 0·8 ml min^{-1} the retention times of the mixed mononitrates, 1,3-glyceryl dinitrate, 1,2-glyceryl dinitrate and nitroglycerin were 6, 10, 11·5 and 26·5 min, respectively. The nitroglycerin prepared by the synthetic/separation process had a radiochemical purity of 98·9% and a specific activity of 0·125 Ci mmol^{-1}.

Tissue preparation. Pieces of liver, lung, leg muscle, vena cava and aorta removed from the three sheep anaesthetized with sodium pentobarbitone, were placed in ice cold 0·1 M phosphate buffer at pH 7·4, weighed and briefly dried on blotting paper. After slicing into small pieces, the tissues were homogenized in 2 volumes of fresh phosphate buffer in a Sorvall blender to form a final tissue suspension.

Tissue metabolic studies. Samples (4 ml) of the sheep liver, lung, leg muscle, vena cava and aorta homogenates were transferred to glass centrifuge tubes in a water bath heated to 37 °C. As recommended by Needleman & Hunter (1965) the co-factors reduced glutathione (8 mM), and NADPH (0·7 mM) were added to the homogenates. After 3 min preincubation of the mixture, tritiated nitroglycerin was added in 100 µl saline to give concentrations of about 40 and 10 ng ml^{-1} suspension. Nitroglycerin was also added to tubes containing buffer and co-factors but no tissue. The possibility of metabolite inhibition of nitroglycerin metabolism was studied by adding 1,3-glyceryl dinitrate and 1,2-glyceryl dinitrate (each at final concentrations of about 100 ng ml^{-1} suspension) to some of the tubes. At specified times, 200 µl samples were removed from the homogenates incubated at 37 °C and the metabolism terminated by the addition of 200 µl methanol. After

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vortexing and centrifugation the samples were stored at −20 °C until analysed.

Glyceryl nitrate analysis. The tissue samples (150 μl) were injected directly onto the HPLC system used for purification of nitroglycerin and the labelled nitroglycerin and metabolites recovered as fractions of the mobile phase effluent. Scintillation cocktail (10 ml) (Biofluor, New England Nuclear, Boston) was added to each vial containing a given fraction and the tritium content determined by liquid scintillation spectrometry (LKB Instruments, Rockville). The metabolites were quantified by assuming they had specific activities per mole identical to that of the labelled nitroglycerin. To ensure good recoveries (97–100%) for the labelled glyceryl nitrates, addition of 5 μl of an aqueous solution containing 10 to 15 μg ml^{-1} of each unlabelled nitrate to the tissue samples before injection was necessary. The addition of unlabelled nitrates also allowed direct visualization of the nitrates eluting from the column via a fixed wavelength detector (214 nm) (Model 441, Waters Associates, NSW). This detector provided the basis for the collection of individual organic nitrate fractions. The volume and timing of a given fraction was based on the appearance of a peak corresponding to the organic nitrate with allowance being made for the small lag between detection and collection times.

Results

Nitroglycerin degradation. Figs 1 and 2 show the time course for the disappearance of nitroglycerin (initial concn, 50 ng ml^{-1}) after incubation with various tissue homogenates. Similar profiles were found after 10 ng ml^{-1} (not shown), the profiles not being significantly different from those in Figs 1 and 2. The disappearance of nitroglycerin from all incubations could be accounted for by the production of its dinitro and mononitro metabolites. Table 1 shows the mean proportion of each species found in tissue homogenates after 30 min of incubation of the initial nitroglycerin concentrations of 50 and 10 ng ml^{-1}.

Effect of glyceryl dinitrates on nitroglycerin metabolism. The presence of 1,3-glyceryl dinitrate and 1,2-glyceryl dinitrate each at concentrations of 100 ng ml^{-1} greatly reduced the rate of nitroglycerin metabolism by all tissue homogenates for the initial nitroglycerin concentrations 50 ng ml^{-1} (Figs 1, 2) and 10 ng ml^{-1} (Table 1).

Discussion

The present work has shown that the various sheep tissues (liver, muscle, artery, vein and lung) rapidly metabolize nitroglycerin to its dinitrometabolites. The results (Table 1) also show that each of the tissues further metabolize the dinitrometabolites to the mononitrate metabolites. The significant lung tissue metabolism of nitroglycerin in the sheep conflicts with the

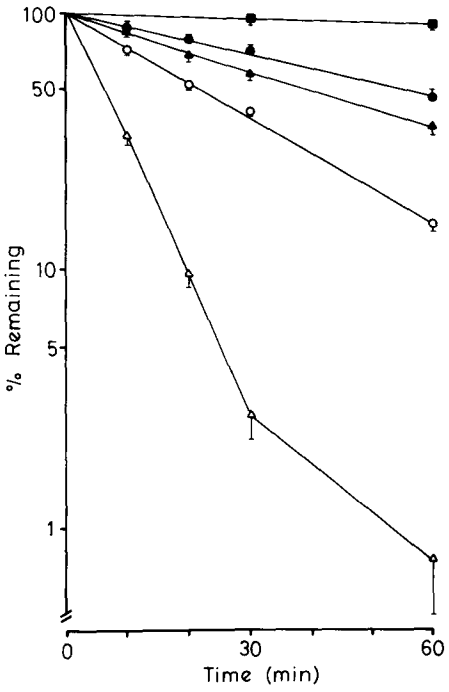

FIG. 1. Disappearance of nitroglycerin (initial concentration 50 ng ml^{-1}) from liver (▲△) and lung (●○) tissue homogenates in the presence of 100 ng ml^{-1} of glyceryl 1,2-dinitrate and 100 ng ml^{-1} glyceryl 1,3-dinitrate (closed symbols) and in the absence of metabolite (open symbols). The disappearance profile in the absence of tissue homogenates (control) are also given (■). (Mean ± s.d., n = 3.)

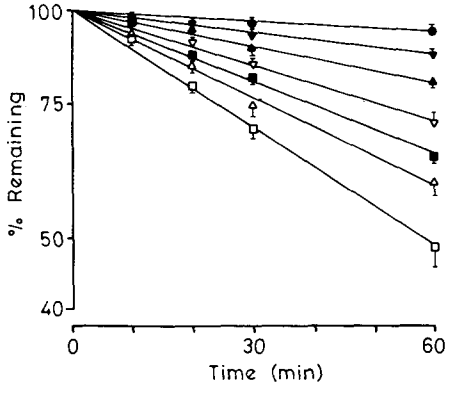

FIG. 2. Disappearance of nitroglycerin (initial concentration 50 ng ml^{-1}) from muscle (■□), vein (▲△) and artery (▼▽) tissue homogenates. Open symbols represent values obtained on incubation without addition of metabolites and closed symbols represent values obtained for incubations in the presence of 100 ng ml^{-1} of glyceryl 1,2-dinitrate and 100 ng ml^{-1} glyceryl 1,3-dinitrate. (Mean ± s.d., n = 3.)

Table 1. Mean percentage[a] of nitroglycerin (GTN) and its metabolites, glyceryl 1,3-dinitrate (1,3-GDN) glyceryl 1,2-dinitrate (1,2-GDN) and glyceryl mononitrate (GMN), in sheep tissue homogenates after incubation for 30 min and the effect of added unlabelled GDNs (each 100 ng ml^{-1}) on the metabolism of GTN.

Drug	Initial concn (ng ml^{-1})	Control		Liver		Lung		Muscle		Vena Cava		Aorta	
		−GDN	+GDN	−GDN	+GDN	−GDN	+GDN	−GDN	+GDN	−GDN	+GDN	−GDN	+GDN
GTN	50	95·3	96·1	2·7	58·2	41·5	72·4	69·1	80·8	73·1	87·7	85·3	88·7
	10	94·9	95·1	2·4	56·5	41·2	73·0	68·0	80·7	73·6	84·7	83·6	87·7
1,3-GDN	50	2·3	2·2	37·2	16·3	17·7	9·7	16·3	10·0	16·3	8·0	7·3	5·9
	10	3·1	3·0	36·8	14·0	17·3	10·4	16·3	11·1	14·9	10·5	8·2	6·0
1,2-GDN	50	2·3	1·7	52·7	25·4	38·0	17·3	13·7	9·1	10·1	4·1	7·1	5·4
	10	2·0	1·8	53·3	28·9	40·0	15·8	15·0	8·4	11·3	4·7	8·2	6·1
GMNs	50	0	0	7·1	0·12	2·1	0·86	0·31	0	0	0	0	0
	10	0	0	5·9	0·30	1·9	0·75	0·48	0	0	0	0	0

[a] Expressed as the percentage of the total radioactivity injected into the HPLC column.

reported negligible organic nitrate reductase activity in rat lung tissue (Maier et al 1980) but support the finding of nitroglycerin metabolites in the effluent of rat isolated perfused lung after infusion of high nitroglycerin concentrations (1 μg ml^{-1}) (Heinzow & Ziegler 1981). The significant metabolism of nitroglycerin by artery and vein tissue is consistent with the observations that rat venous and arterial tissue also metabolize nitroglycerin at a rapid rate (Fung et al 1984). It is therefore apparent that the 17% extraction of nitroglycerin across the pulmonary bed and 61% extraction across the arteriovenous bed in man (Armstrong et al 1982) is due in part to the metabolism of nitroglycerin by the various tissues in the bed.

The concentrations of the dinitrates in the plasma of patients receiving continuous infusions or transdermal nitroglycerin have yet to be reported. The concentrations of 100 ng ml^{-1} glyceryl dinitrates are of the order found in sheep after intravenous infusion of nitroglycerin and are about 5 to 10 times the corresponding steady state nitroglycerin concentrations (unpublished observations). Cossum & Roberts (1985) have previously shown that erythrocytes metabolize nitroglycerin by parallel saturable and first order 'enzymatic' processes. The present data (Table 1) suggest that, at low concentrations, nitroglycerin is metabolized in a concentration-independent manner. Addition of large concentrations of the dinitrometabolites appear to cause a saturation of one of the mechanisms which nitroglycerin is denitrated (Table 1).

The immediate implication of the inhibition of nitroglycerin metabolism by metabolites is a possible alteration in nitroglycerin disposition during chronic therapy. However, metabolite inhibition of nitroglycerin metabolism may have further ramifications. Sutton & Fung (1984), in observing the inhibitory effects of the isosorbide mononitrates on isosorbide dinitrate metabolism, raise the possibility that metabolite inhibition may occur in the biophase, creating a change in the concentration/response relationship as a function of duration of therapeutic use.

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