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COMPARATIVE EFFECTS OF GLYCERYL TRINITRATE ON VENOUS AND ARTERIAL SMOOTH MUSCLE *in vitro;* RELEVANCE TO ANTIANGINAL ACTIVITY

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1 A quantitative *in vitro* study has been made of the actions of glyceryl trinitrate and sodium nitrite on vascular smooth muscle (dog femoral artery and saphenous vein; rat portal vein); these have been compared with the actions of papaverine, isoprenaline, salbutamol, pentaerythritol tetranitrate and trimetazidine.

2 Glyceryl trinitrate was more active on the saphenous vein than on the femoral artery in inhibiting noradrenaline and potassium-induced tone.

3 Unlike glyceryl trinitrate, sodium nitrite and isoprenaline, papaverine and diazoxide inhibited noradrenaline-induced contractions of venous and arterial smooth muscle to the same extent.

4 The selective dilator effects of glyceryl trinitrate on venous smooth muscle may explain its action in alleviating the pain of angina pectoris. It is suggested that the use of these three vascular smooth muscle preparations (arterial, and veins with and without spontaneous myogenic activity) is a useful initial screening procedure for prospective antianginal drugs acting by venodilatation.

Introduction

It is nearly a century since William Murrell suggested that glyceryl trinitrate (nitroglycerin) would be effective in the treatment of angina pectoris. He deduced that 'from a consideration of the physiological action of the drug and more especially from the similarity existing between its general action and that of nitrite of amyl . . . it would probably prove of service in the treatment of angina pectoris' (Murrell, 1879). From his clinical experience he concluded, 'I am happy to say that this anticipation has been realised'. Like Lauder Brunton, Murrell felt that the main effect was on the peripheral vessels. Recent clinical studies have tended to confirm this conclusion. The abnormally increased left ventricular filling pressure, end-diastolic volume, ventricular dimensions and myocardial wall tension, which occur at the height of an anginal attack, are all reduced by nitroglycerin (Williams, Glick & Braunwald, 1965; Lee, Sung & Zaragoza, 1970; Parker, 1972). These effects can be explained (see reviews by Parker, 1972, and by Parratt, 1974; 1975) by dilatation of peripheral capacitance vessels.

If this is true, then it ought to be possible to show preferential dilator effects with nitroglycerin and related compounds on isolated venous, as compared to arterial, smooth muscle; it would then be feasible to screen large numbers of prospective antianginal drugs using simple, inexpensive, isolated vascular smooth muscle preparations. Such drugs, like nitroglycerin itself (Hirshfeld, Borer, Goldstein, Barrett & Epstein, 1974; Smith, Redwood, McCarron & Epstein, 1974) might also be effective in reducing the severity and extent of myocardial damage (infarction) which results from occlusion of a coronary artery. It was with these possibilities in mind that the following experiments were carried out.

Methods

Isolated femoral artery and saphenous vein of the dog

The preparations were obtained from anaesthetized greyhounds (20–35 kg) after experiments, at the Western Infirmary, involving the effect of hyperbaric oxygen on ischaemic cardiac muscle blood flow (Ledingham, Marshall & Parratt, unpublished). Lengths of femoral artery and saphenous veins, free from major branches, were removed and stored overnight in Krebs solution at 2–3°C. In order to examine the effects of drugs on isolated vascular smooth muscle, thin transverse sections were cut once to produce strips, each of which was suspended in a

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10 ml organ bath containing Krebs solution (composition: mM: NaCl 118, KCl 4.4, NaH_2PO_4 25, $MgCl_2$ 1.25, $CaCl_2$ 5.4 and glucose 5.5) at 37°C. The solution was gassed with a mixture of 5% CO_2 in O_2. Contractions (of circular smooth muscle layers) were recorded isometrically with Ugo-Basile strain-gauges. Devices pre-amplifiers and a two-channel recorder. Arterial and venous preparations from the same dog were studied simultaneously. The resting tension was 0.5 gram. The femoral artery and saphenous vein strips had very little resting tone and were therefore contracted with either noradrenaline (2–10 μM) or potassium (K^+; 50–100 mM). Submaximal (60–80% of maximum) contractions induced by these agonists were maintained and reproducible. Sodium nitrite, glyceryl trinitrate and the other drugs mentioned in the Results section, were added to the bath in increasing concentrations, without washing, and the degree of inhibition of the noradrenaline or K^+-induced tone was assessed.

Isolated portal vein of the rat and dog

Portal veins were removed from rats weighing between 200 and 500 g and were immersed in Krebs solution at a temperature of 37°C. A tension of 0.5 g was applied to the tissue and the contractions recorded isometrically. Longitudinal strips prepared from the portal vein of the dog were also used. Both preparations exhibited inherent, spontaneous, myogenic activity, and inhibition of this activity by the cumulative addition of various drugs was examined.

The drugs used were: alprenolol (Hassle), atropine, glyceryl trinitrate (Evans Medical), (−)-isoprenaline (Wyeth), (−)-noradrenaline (Sigma), salbutamol (Allen & Hanbury), sodium nitrite, pentaerythritol tetranitrate (Warner) and trimetazidine (Servier).

Results

Effects of sodium nitrite

Sodium nitrite relaxed all three types of vessels studied (Figure 1). The threshold concentration (100 μM), and the slope of the dose-response curves up to approximately 40% inhibition (approximately 1 mM; Figure 1) were similar. Concentrations in excess of 1 mM continued to inhibit the tone of the saphenous and portal veins up to 80% inhibition (10 mM; the highest concentration used in the rat portal vein). Complete inhibition of induced tone was produced in the femoral vein with 30 mM of sodium nitrite. In contrast to the effects on the venous preparations, there was no further reduction in arterial tone with increasing concentrations of sodium nitrite (from 1 to 10 mM); concentrations in excess of 10 mM increased the tone such that, at the highest concentration (100 mM), it was only 10% less than the original

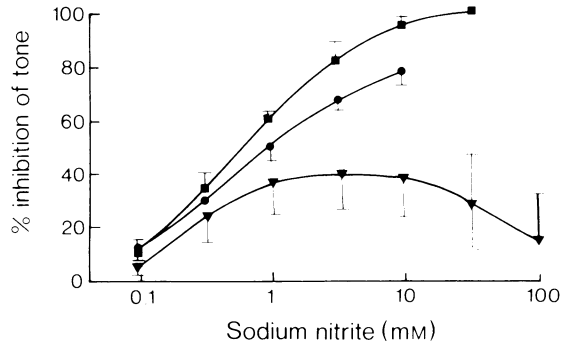

Figure 1 The inhibitory effect of sodium nitrite on the inherent myogenic tone of the rat portal vein (●) and on tone induced by noradrenaline (2–10 μM) in the dog saphenous vein (■) and femoral artery (▼). Note the unusual shape of the dose-response curve for sodium nitrite on the femoral artery. Each point represents the mean of five to six observations. Vertical lines show s.e. mean.

control level. An example of such an experiment is shown in Figure 2. This response is presumably due to the high concentration of sodium ions releasing calcium from intracellular stores.

Effects of glyceryl trinitrate

Initially, glyceryl trinitrate solutions were made from tablets but in the later experiments dilutions were made, in distilled water, from a stock solution of glyceryl trinitrate contained in alcohol. Because alcohol itself relaxed the vascular smooth muscle preparations used, solvent controls were carried out in all experiments. No strength of glyceryl trinitrate was used which contained greater than one-tenth the concentration of solvent which itself produced a response. The results with glyceryl trinitrate obtained from both sources (tablets and alcoholic solution) were identical.

The responses to glyceryl trinitrate were similar to those of sodium nitrite (Figure 3). In the femoral vein complete inhibition was obtained (at 50 μM; five preparations) without approaching the threshold to the alcohol response. In contrast, the femoral artery could only be inhibited by about 50%, even with concentrations in excess of 5 mM. The threshold dose for the artery was about 0.4 μM, i.e. 10 times that for the vein (Figure 3).

In order to determine whether the glyceryl trinitrate-induced relaxation of the femoral vessels was due to a specific antagonism of noradrenaline, the experiments were repeated using potassium instead of noradrenaline to induce contracture. The threshold concentration of glyceryl trinitrate was similar (0.4 μM) in these experiments, with maximum inhibition (60%) of the artery occurring at a concentration of 50 μM (five preparations) and of the vein

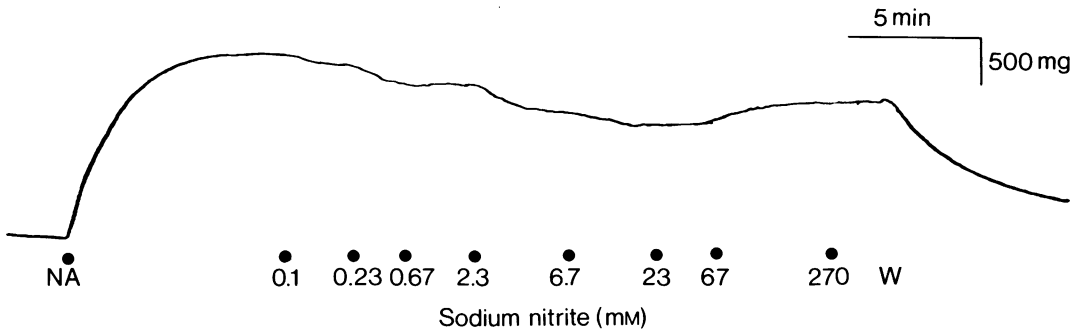

Figure 2 The inhibitory effect of cumulative additions of sodium nitrite on the tone induced by noradrenaline (NA, 2 μM) in the dog femoral artery. Note the increase in tone produced by concentrations of sodium nitrite in excess of 100 μM. W = wash.

(100% inhibition) at 500 μM (six preparations). The degree of inhibition of the femoral vein (100%) and artery (55–60%) was the same against a potassium-induced contracture as against one induced by noradrenaline (Figure 3) although higher concentrations of glyceryl trinitrate had to be used.

Inhibition of noradrenaline-induced contraction by glyceryl trinitrate and sodium nitrite was unaffected by pretreatment with atropine (1.4 μM), alprenolol (1.8 μM) or mepyramine (3.5 μM). These concentrations were sufficient to antagonize the actions of acetylcholine, isoprenaline or histamine respectively.

Effects of other vasodilator drugs

The fact that sodium nitrite and glyceryl trinitrate preferentially relaxed venous smooth muscle could be explained by simple ease of drug access, in the thinner venous preparations, to the receptors involved. That this is not the explanation is clear from Figure 4 which shows the comparative effects of papaverine and diazoxide on saphenous vein and femoral artery preparations. Both drugs inhibited noradrenaline-induced contractions to the same extent in the two preparations.

Some indication of the relative effectiveness of glyceryl trinitrate was obtained by comparison of its effects on all three preparations with those of isoprenaline (Figure 5) and, on the rat portal vein, with salbutamol and other antianginal agents, pentaerythritol tetranitrate and trimetazidine. The results were summarized in Figure 6.

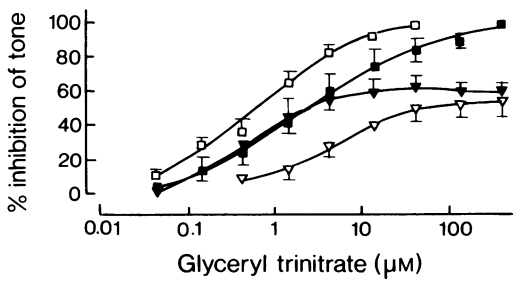

Figure 3 A comparison of the effect of glyceryl trinitrate on tone induced by noradrenaline (open symbols) and by potassium (closed symbols) in the dog femoral artery (▽▼) and saphenous vein (□■). Each point represents the mean of at least five observations. Vertical lines show s.e. mean.

Discussion

The results obtained with the dog isolated vessels indicate that both glyceryl trinitrate and sodium nitrite

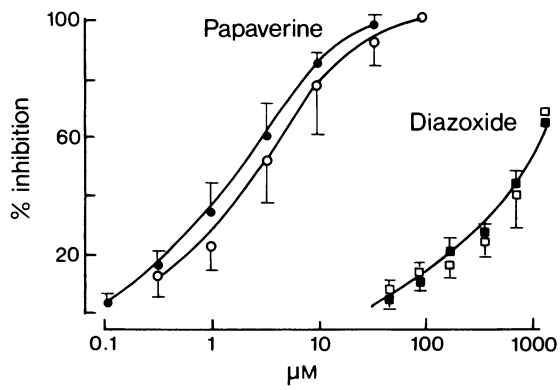

Figure 4 Comparative effects of papaverine and of diazoxide on tone induced by noradrenaline in the dog femoral artery (○□) and saphenous vein (●■). Each point represents the mean of five to six observations. Vertical lines show s.e. mean. In contrast to the effect of glyceryl trinitrate, both these drugs inhibited arterial and venous smooth muscle preparations to the same extent, i.e. they showed no particular selectivity for venous smooth muscle.

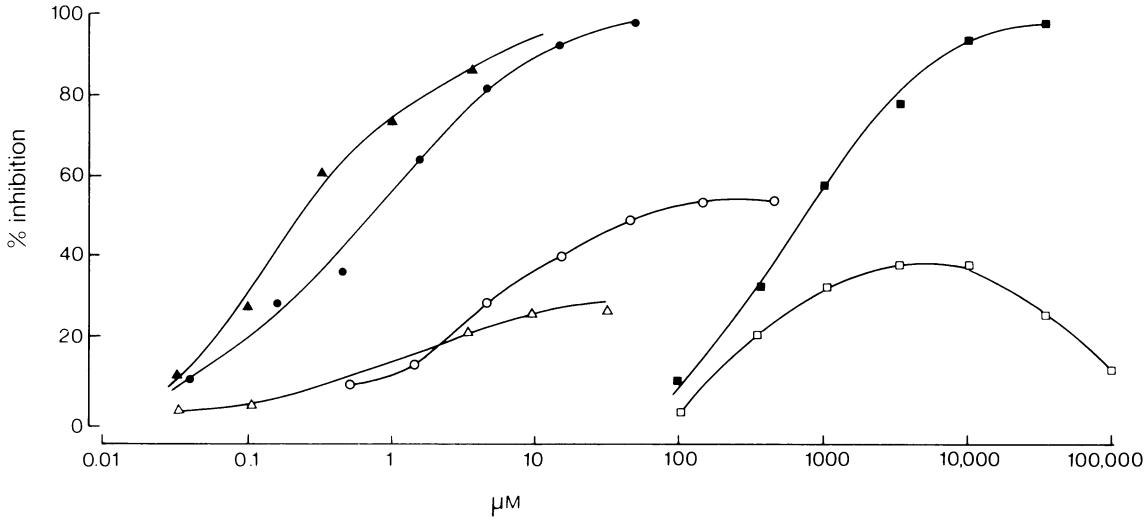

Figure 5 The effects of isoprenaline (▲△) glyceryl trinitrate (●○) and sodium nitrite (■□) on dog saphenous veins (closed symbols) and femoral arteries (open symbols) contracted with noradrenaline (2–10 μM). Each point represents the mean of six observations. All three agents inhibit venous smooth muscle more than they do arterial smooth muscle, i.e. they demonstrate selectivity for venous smooth muscle.

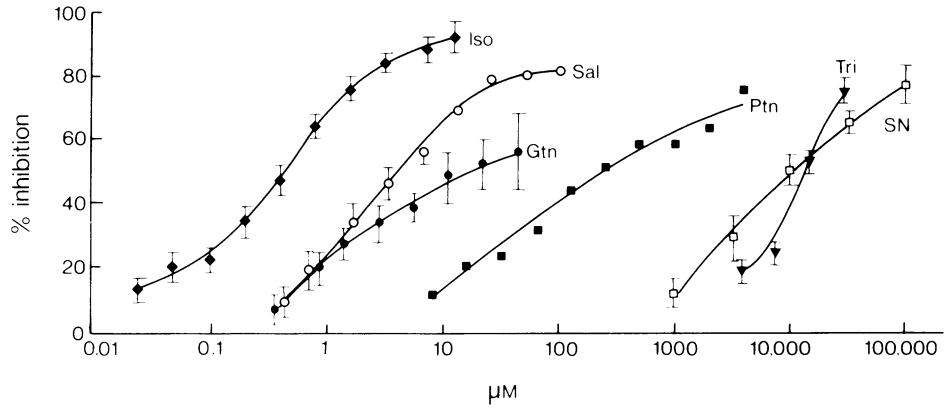

Figure 6 Comparison of the effect of isoprenaline (Iso), salbutamol (Sal), glyceryl trinitrate (Gtn), pentaerythritol tetranitrate (Ptn), trimetazidine (Tri) and sodium nitrite (SN) on spontaneous myogenic activity of rat portal veins.

preferentially relax venous smooth muscle, especially when it is contracted with noradrenaline. For example, at a concentration of 1 μM glyceryl trinitrate inhibited, by more than 50%, noradrenaline-induced tone in the saphenous vein whereas this concentration had only a slight (<10%) effect on noradrenaline-induced tone in the femoral artery. At all concentrations glyceryl trinitrate was significantly ($P < 0.001$) more effective on the saphenous vein than on the femoral artery. Even in concentrations in excess of 100 μM it could only inhibit tone in arterial preparations by 50%. In contrast, noradrenaline-induced tone in venous smooth muscle was completely inhibited by a concentration of only 10 μM. A similar differential effect on the two smooth muscle preparations was also obtained with isoprenaline, which was equiactive with glyceryl trinitrate, and with sodium nitrite, which was 1000 times less active. This indicates that nitroglycerin does not have to be reduced from the ester to the nitrite ion before it becomes an active venodilator and also that only relatively small amounts of circulating nitroglycerin are required to dilate veins in which tone

is increased with noradrenaline. An increased sensitivity of peripheral veins to this neurotransmitter has been recently demonstrated in anginal patients by Robinson (1975).

This selectivity for venous smooth muscle is not due to the relative thinness of the walls of the veins allowing ease of drug access since papaverine and diazoxide showed no such selectivity for venous smooth muscle. In fact papaverine was active in a similar concentration to glyceryl trinitrate and isoprenaline on the noradrenaline-contracted saphenous vein but was much more active than either drug on the femoral artery.

The results obtained from these relatively simple *in vitro* experiments are in general agreement with those obtained with more sophisticated *in vivo* models. Using the Mellander (1960) technique for simultaneously assessing changes in the responses of series-coupled resistance and capacitance vessels, and on precapillary sphincter activity in the cat hindquarters, Johnsson & Öberg (1968) found that nitroglycerin and sodium nitrite had more marked effects on capacitance vessels. On the other hand, isoprenaline (Johnsson & Öberg, 1968) and hydralazine (Ablad & Mellander, 1963) had more pronounced dilator effects on precapillary resistance vessels; this, by decreasing the pre- to post-capillary resistance ratio, favoured transcapillary exchange. There are studies on the human forearm that also indicate that the main site of action of nitroglycerin is on capacitance vessels (Mason & Braunwald, 1965; Williams *et al.*, 1965). The resultant venous pooling and reduced return of blood to the right side of the heart, together with pulmonary vasodilatation (Cyong, Tanaka, Horiguchi, Tsuchiya & Itoh, 1976), would adequately explain the decreased ventricular filling pressure, end-diastolic volume and heart size, and the resultant reduction in myocardial oxygen consumption, reported to occur in anginal patients after nitroglycerin (see Introduction).

Whilst papaverine was able to abolish completely noradrenaline-induced tone in both arterial and venous tissues, the maximum inhibition of tone produced by glyceryl trinitrate was not the same, being much less in arterial than in venous smooth muscle (Figure 3). Furthermore this difference was independent of the means by which tone was induced (i.e. with noradrenaline or potassium, Figure 3). Similar degrees of inhibition were produced by isoprenaline (Figure 5). An additional similarity between isoprenaline and glyceryl trinitrate is that they are both potentiated by sub-inhibitory concentrations of phosphodiesterase inhibitors (Levy & Wilkenfeld, 1968). Isoprenaline relaxes vessels by β-adrenoceptor activation which results in stimulation of adenyl cyclase and the production of cyclic adenosine 3′,5′-monophosphate (cyclic AMP); this leads to Ca^{2+} sequestration and relaxation (Andersson, 1973). Phosphodiesterase inhibitors potentiate this action of isoprenaline by preventing the breakdown of cyclic AMP to AMP. It has been shown that glyceryl trinitrate is not itself a phosphodiesterase inhibitor (Levy & Wilkenfeld, 1968; Poch & Kukovetz, 1972) and the present work shows that an effect on β-adrenoceptors is not involved since its actions were unaffected by alprenolol. The similarity between the two drugs could be explained by glyceryl trinitrate stimulating adenyl cyclase either directly, or by a process not involving β-adrenoceptors. The release of bound Ca^{2+} by agonists would then be prevented as a result of the increased levels of cyclic AMP by an action of nitroglycerin at a site distal to the β-adrenoceptor but proximal to that of phosphodiesterase inhibition. The difference in maximum inhibition of veins and arteries could then be due to a difference in the maximum cyclic AMP levels attainable.

One approach to more effective anginal therapy is to produce drugs that have a similar basic mode of action to nitroglycerin. The present studies have emphasized that this mechanism is a selective dilator action on peripheral veins. We suggest therefore that a simple primary screen for new antianginal compounds would be to examine their relative activity on isolated arteries and veins (contracted with noradrenaline or potassium) and on isolated veins with spontaneous myogenic activity (e.g. portal vein of the rat). Such experiments would not of course give any indication of effects on the microcirculation; they should however indicate which compounds would be worth pursuing using more sophisticated tests for antianginal activity (Parratt, 1974).

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