*Br. J. clin. Pharmac.* (1981), **1**, 57–61

# A COMPARISON OF THE EFFECTS OF HYDRALLAZINE, DIAZOXIDE, SODIUM NITRITE AND SODIUM NITROPRUSSIDE ON HUMAN ISOLATED ARTERIES AND VEINS

R.F.W. MOULDS, R.A. JAUERNIG* & J. SHAW†

Department of Medicine, University of Melbourne,
The Royal Melbourne Hospital, Parkville, Victoria 3050, Australia

1 Human common palmar digital arteries and dorsal metacarpal veins have been studied *in vitro* to investigate the responses of arterial and venous muscle to hydrallazine, diazoxide, nitroprusside and sodium nitrite.

2 Tissues were removed at autopsy, cut into helical strips and suspended in organ baths under identical conditions. The contractile response to noradrenaline was tested in the presenceof the same concentrations of the vasodilator drugs in arteries and veins.

3 Hydrallazine antagonised contraction of arteries, but not veins to noradrenaline. Diazoxide, nitroprusside or sodium nitrite antagonised responses in both arteries and veins. Diazoxide and nitrite were more effective on arteries and nitroprusside was more effective on veins.

4 These results are in accord with clinical observations and confirm that there are differences in the susceptibility of human arterial and venous smooth muscle to vasorelaxant drugs.

## Introduction

Drugs that have a direct relaxant effect on vascular smooth muscle have long been used in clinical medicine. Hydrallazine has been available as an antihypertensive agent for over 20 years, nitroprusside and diazoxide are widely used for hypertensive emergencies and nitrites are used for the relief of angina pectoris.

Vasodilator drugs have a beneficial effect on congestive cardiac failure (Chatterjee & Parmley, 1977; Cohn & Franciosa, 1977). Clinical studies show differing responses to these agents which are attributed to different patterns of activity on the venous and arterial vascular beds. Accordingly, their predominant mode of action may be either by reducing cardiac preload and/or vascular resistance. However, it is not clear whether this is due to different sensitivities of the two vascular beds to vasorelaxant drugs or merely to small changes in capacitance vessels.

We have recently described a human arterial preparation for studying *in vitro* the effects of vasoactive agents (Jauernig & Moulds, 1978), and have used this and an identically treated venous preparation to demonstrate a greater effect of the $\alpha$-adrenoceptor antagonist prazosin on venous than arterial preparations (Jauernig, Moulds & Shaw, 1978). We have therefore extended these studies to compare and contrast the actions of hydrallazine, sodium nitrite, sodium nitroprusside and diazoxide on human vascular smooth muscle.

Present addresses:
* Department of Cardiology, Medizinische Universitatäts-Klinik, Bergheimer Strasse 58, 6900 Heidelberg, West Germany.

† Department of Clinical Pharmacology, University of Sydney, Sydney, New South Wales 2006, Australia.

## Methods

The blood vessels used for this study were the common palmar digital arteries of the second and third fingers, and the dorsal metacarpal veins, usually obtained from the same patient. The vessels were obtained at autopsy, usually less than 24 h after death. They were cut into spiral strips approximately 1.5 cm long and suspended separately in tissue baths which contained 30 ml bathing fluid of the following composition (mmol $l^{-1}$): $Na^+$ 137.4, $K^+$ 5.4, $Ca^{2+}$ 2.5, $Cl^-$ 131.5, $SO_4^{2-}$ 1.2, $H_2PO_4^-$ 1.2, $HCO_3^-$ 15.0, glucose 11.5 and gassed with 95% $O_2$ and 5% $CO_2$. The initial resting tension was adjusted to approximately 1 g, and muscle responses were measured using Grass FT 03 C and Hewlett Packard FTA 100–1 isometric force transducers and a Hewlett Packard 7758 A and 7702 B recorder. The metacarpal veins were treated identically to the arteries except

0306–5251/81/010057–05 $1.00

that the resting tension was readjusted during the initial period of relaxation in the tissue bath because the veins had a greater tendency to relax after being suspended in the tissue bath. The final resting tension for both arteries and veins was thus approximately 1 g.

Because the preparations were already completely relaxed, the effect of the vasodilating agents was assessed by measuring their inhibitory effects against contractions to noradrenaline. Cumulative concentration-effect curves to noradrenaline were obtained in the absence and in the presence of three concentrations of the vasodilators. Only the effect of one vasodilator was tested on each preparation. In all cases, control concentration-effect curves to noradrenaline (i.e. in the absence of other drugs) were repeatedly performed on a different preparation from the same blood vessel throughout the experiment, and the responses to noradrenaline in the experimental strips were corrected for any tachyphylaxis occurring in the control strips. In order to compare the maximum responses to noradrenaline in arteries and veins, responses were expressed as a percentage of the contractile response to 80 mmol $l^{-1}$ potassium chloride (tested at the beginning of each experiment).

The isometric force was plotted against the noradrenaline concentration and typical sigmoid dose response curves were obtained. As the effects of the various drugs on the noradrenaline dose-response curves were neither those of classical competitive nor non-competitive inhibition the results were analysed in the following ways.

First, the reduction of the maximum response to noradrenaline was expressed as a percentage of the initial maximum response. Secondly, for each vessel tested a level of contraction was sought where, over the range of antagonist concentrations used, the curves were close to parallel; 40% of the initial maximum contraction was usually suitable. The concentration of noradrenaline required to produce the level of contraction in the absence and in the presence of the antagonist was then calculated from the graphs and expressed as the noradrenaline dose-ratio (Figure 1). The mean of the logarithm (base 10) of the noradrenaline dose-ratios was then calculated for each concentration of each antagonist.

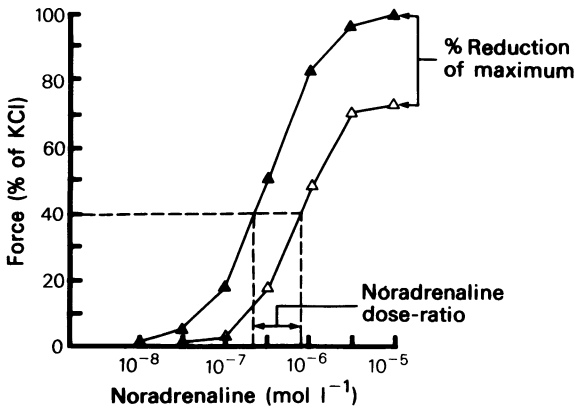


**Figure 1** Illustration of the method used to calculate the inhibitory effect of the vasodilator drugs on the response to noradrenaline ▲ control concentration-effect curve to noradrenaline. △ concentration-effect curve to noradrenaline repeated in the presence of the vasodilator drug.

With each drug, the same three concentrations were tested in both arteries and veins, and a 125-fold range of concentrations was used.

Results were compared using a two-tailed Student's paired *t*-test when all arteries and veins had been taken from the same patient. In other cases Student's unpaired *t*-test was used.

Drugs used were noradrenaline (l-arterenol bitartrate, Sigma), sodium nitroprusside (David Bull Laboratories), diazoxide (Hyperstat, Schering), hydrallazine hydrochloride (Apresoline, Ciba-(Geigy) and sodium nitrite (AnalaR). The drugs were prepared in distilled water alone, or (for noradrenaline) containing ascorbic acid 100 $\mu$mol $l^{-1}$.

## Results

The arterial preparation used has been previously described in some detail and has been shown to provide reproducible results with a number of pharmacological agonists and antagonists (Jauernig & Moulds, 1978; Jauernig, Moulds & Shaw, 1978; Moulds, Jauernig, Hobson & Shaw, 1978). The sensitivity of the preparation is unrelated to the time elapsed since death, up to at least 24 h. The venous preparations gave reproducible responses in a similar manner to the arterial preparations over periods of at least 6–8 h. The time after death, from 2–24 h, did not significantly affect the responses of the venous preparation.

Hydrallazine, diazoxide, nitroprusside and sodium nitrite, antagonised the effects of noradrenaline in a concentration-dependent manner, but the pattern of potencies was different for each drug.

### 1. *Nitroprusside*

Nitroprusside produced a near-parallel shift of the noradrenaline dose-response curves in both arteries and veins (Figure 2). The full dose-response curves have been shown in Figure 2 as an example of the effects of one of the vasodilators. For analysis of the results in each case one of the doses of vasodilator has been selected, and for nitroprusside $4 \times 10^{-8}$ mol $l^{-1}$

produced a significantly greater shift of the noradrenaline dose-response curve ($P<0.05$, paired $t$-test) in the arteries than in the veins (Figure 3). The same concentration of nitroprusside reduced the maximum response to noradrenaline to a similar degree in both arteries and veins (Figure 4).

2. *Hydrallazine*

The arterial preparations were variably affected. Increasing concentrations of hydrallazine shifted the dose-response curves to the right in a near-parallel manner and caused a small reduction of the maximum response. In the veins, the same concentrations of hydrallazine did not shift the curves. The mean shift of the curves produced by hydrallazine ($2 \times 10^{-5}$ mol $l^{-1}$) was greater ($P<0.05$) in the arteries than in the veins (Figure 3). In both arteries and veins, the mean maximum response to noradrenaline was slightly reduced by the same concentration of hydrallazine (Figure 4).

3. *Diazoxide*

In the arteries, diazoxide ($5 \times 10^{-5}$ mol $l^{-1}$) produced a clearly non-parallel shift of the noradrenaline dose-response curves and a marked reduction of the maximum response. As a result dose-ratios for

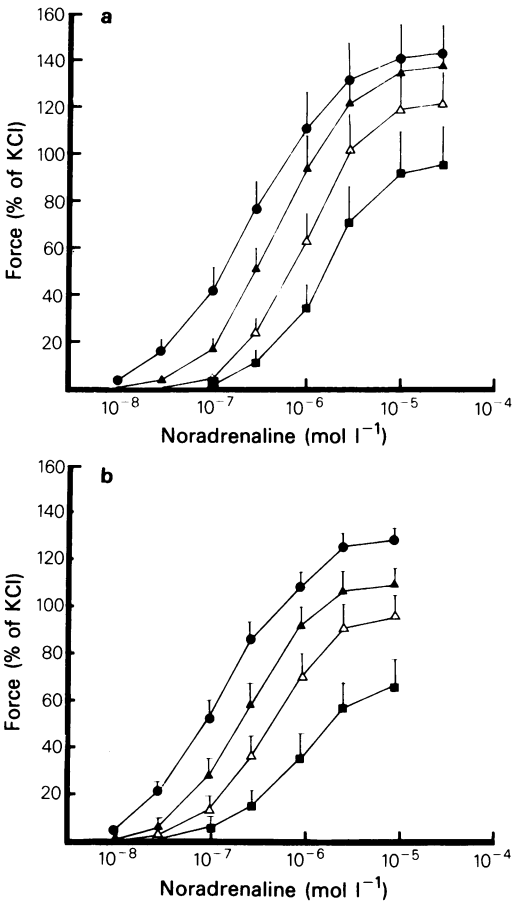


**Figure 2** Concentration-effect curves to noradrenaline in arteries (a) and veins (b) in the absence of (●) and in the presence of increasing concentrations of nitroprusside:

▲ $8 \times 10^{-9}$ mol $l^{-1}$,
△ $4 \times 10^{-8}$ mol $l^{-1}$,
■ $2 - 10^{-7}$ mol $l^{-1}$.

The results shown are the mean (with bars showing s.e. mean) of the responses of ten arteries with ten veins.

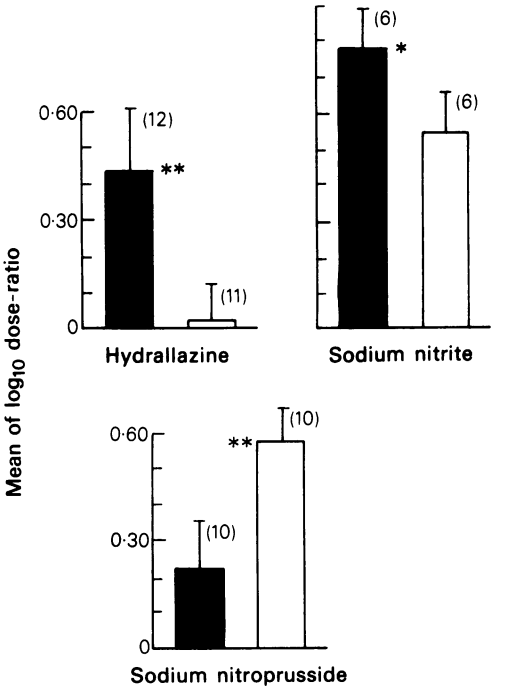


**Figure 3** Means (with bars showing s.e. mean) of the logarithms of the dose-ratios of noradrenaline produced by the vasodilators on arteries ( ■ ) and veins ( □ ).

The concentrations of each of the vasodilators illustrated are hydrallazine, $2 \times 10^{-5}$ mol $l^{-1}$; sodium nitrite $2.5 \times 10^{-4}$ mol $l^{-1}$; and sodium nitroprusside $4 \times 10^{-8}$ mol $l^{-1}$.

Difference between arteries and veins,
* $P<0.05$ , ** $P<0.01$

The figures in parentheses show the numbers of vessels tested.

noradrenaline could not be calculated. In the veins, the same concentration of diazoxide produced a more parallel shift of the noradrenaline dose-response curve, and the reduction of the maximum response was significantly smaller than in arteries ($P<0.05$, Figure 4).

4. *Sodium nitrite*

Sodium nitrite caused near-parallel, concentration-dependent shifts of the noradrenaline dose-response curves in both arteries and veins and reduced the maximum responses to a similar extent (Figure 4). However, the mean dose-ratio for noradrenaline in the presence of the highest concentration of nitrite ($2.5 \times 10^{-4}$ mol $l^{-1}$) was slightly, but significantly, higher ($P<0.05$, paired $t$-test) in the arteries (Figure 3).

## Discussion

This study has confirmed that there are differences in the susceptibility of human arterial and venous smooth muscle to vasodilating agents. It is unlikely that the differences described here are due to methodological factors, since different drugs have shown a preferential action on either arterial or venous smooth muscle. Moreover, except with sodium nitrite, the patterns are similar to those that have been inferred from *in vitro* studies (Collier, Lorge & Robinson, 1978).

Our results support the view (Ablad, 1963) that hydrallazine has a direct vasodilator action predominantly on the arterial vascular bed. In comparison to the other drugs tested, the effects of hydrallazine were generally small and variable in both arteries and veins, even though the range of concentrations tested was the same for all four drugs. However, hydrallazine demonstrated the greatest difference between arterial and venous responses in that veins were almost completely resistant to its effects.

Diazoxide has previously been shown to be a vascular muscle relaxant in animal preparations (Wohl, Hausler & Roth, 1968; Rhodes & Sutter, 1971). *In vivo* studies suggest that diazoxide acts predominantly on the arterial side of the vascular tree. Our study confirms this, but shows that the drug also has a considerable effect on veins.

Differences between various vascular beds in their sensitivity to sodium nitroprusside has previously been found in animal studies (Kreye, Baron, Lüth & Schmidt-Gayk, 1975; Verhaeghe & Shepherd, 1976). Our study also shows a significantly greater effect on veins. However, since there was a marked effect on the arteries, both arterial and venous vascular beds are probably affected in the therapeutic use of nitroprusside.

Higher potency of nitrite in arteries rather than in the veins disagrees with the previous report of Collier *et al.* (1978), but is consistent with the clinical observation that in the treatment of congestive cardiac failure, intravenous infusion or repeated oral use of glyceryl trinitrate produces similar results to those of hydrallazine, which was also found to be more potent in inhibiting the responses of arteries than veins.

Care must always be exercised in extrapolating from the *in vitro* to the *in vivo* situation. The sites of drug action may not be identical in the *in vivo* and *in vitro* situations, and the blood vessels we used may not represent the entire circulatory system. Thus, differences in sensitivities of other vascular beds, degrees of sympathetic tone, and rates of drug metabolism are among the many factors which may complicate the interpretation of the results. Nevertheless, the *in vitro* data gives strong evidence for differential drug actions on arteries and veins: previously this has been assumed or concluded only from indirect parameters.

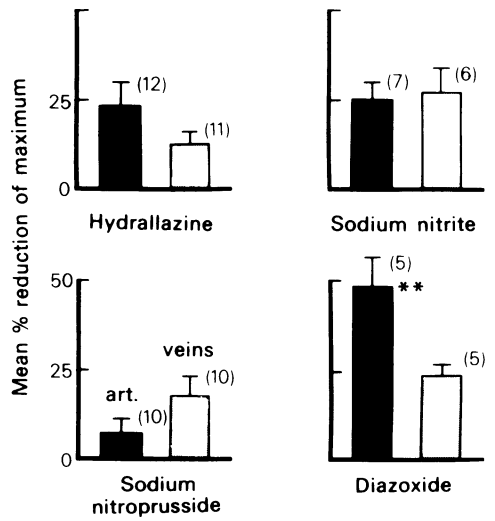


**Figure 4** Means (with bars showing s.e. mean) of the per cent reduction of the maximum response produced by the vasodilators on arteries ( ■ ) and veins ( □ ).

The concentrations of the vasodilators are the same as in Figure 2, and the diazoxide concentration illustrated is $5 \times 10^{-5}$ mol $l^{-1}$.

** $P<0.01$ for difference between arteries and veins.

The figures in parentheses show the number of vessels tested.

The excellent technical assistance of Miss C. Woodroofe and Mr P. Worland is gratefully acknowledged.

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*(Received August 28, 1979, revised September 3, 1980)*