Simultaneous perfusion of rat isolated superior mesenteric arterial and venous beds: comparison of their vasoconstrictor and vasodilator responses to agonists

Timothy D. Warner

The William Harvey Research Institute, The Medical College of St. Bartholomew's Hospital, Charterhouse Square, London, EC1M 6BQ

1 A new isolated perfused preparation is described that allows a direct comparison to be made of the responses of the perfused arterial and retrogradely perfused venous circulations of the rat superior mesenteric vascular bed.

2 In experiments comparing the responses of the intact arterially perfused mesentery and small intestine to those of the same preparation following removal of the intestine and division of the circulations, the increases in perfusion pressure produced by arginine-vasopressin (30 pmol) and noradrenaline (1 nmol) were retained by the arterial circulation and those induced by angiotensin II (30 pmol) by the venous circulation. Endothelin-1 (30 pmol) constricted both portions of the vasculature but the prolonged nature of its response was associated with only the venous vessels.

3 In the simultaneously perfused arterial and venous preparation arginine vasopressin (3–100 pmol) was a selective constrictor of the arterial circulation and angiotensin II (3–100 pmol) of the venous circulation. In addition, noradrenaline (0.3–10 nmol), 5-hydroxytryptamine (0.3–10 nmol) and KCl (1–60 μ mol) were more active as constrictors of the arterial than the venous vessels, and U46619 (10–300 pmol) a more active constrictor of the venous than the arterial vessels. Endothelin-1 (3–100 pmol) constricted both the arterial and venous portions of the vasculature but was significantly longer acting as a venoconstrictor than an arterioconstrictor.

4 Angiotensin I (300 pmol) caused constrictions of the venous circulation which were dependent upon the presence of angiotensin converting enzyme for captopril (10 μ M) abolished constrictions caused by angiotensin I but not by angiotensin II.

5 In preparations preconstricted by U46619 (0.3–3 μ M), acetylcholine (0.01–100 nmol), bradykinin (0.001–1 nmol), sodium nitroprusside (0.01–10 nmol) or isoprenaline (1–100 pmol) produced dose-related dilatations of both the arterial and the venous vasculatures, whereas adenosine diphosphate (ADP, 0.01–100 nmol) caused dose-dependent dilatations of the arterial circulation but principally constrictions of the venous circulation. The dilatations caused by acetylcholine and bradykinin in both portions of the circulation, and by ADP in the arterial circulation, were endothelium-dependent as they were inhibited by gossypol (3 μ M), whereas dilatations to sodium nitroprusside were not.

6 This preparation allows the responses of the arteries and veins of a single perfused mesenteric bed to be compared. In addition, with this preparation it is possible to demonstrate that veins, as well as arteries, show significant endothelium-dependent relaxations. It is concluded that the venous portion of the vasculature is significantly involved in the responses of the intact circulation.

Introduction

The venous compartment of the vasculature is crucial to the functioning of the circulation as a whole as it can affect both cardiac preload and capillary filtration (Folkow & Mellander, 1964). However, the study of venous function has always been second to that of arterial function. This is in part due to the assumption that the responses of arteries are more important than those of veins. However, it must also be because of the methodological problems involved in the study of the venous circulation. The veins are by their nature more difficult for isolated tissue studies than arteries, while *in vivo* the responses of the veins have to be interpreted indirectly from other measures. In this paper a retrogradely perfused venous preparation is described that allows direct comparisons to be made between the responses of the veins and arteries within the isolated perfused superior mesenteric vascular bed of the rat.

Methods

Double perfused mesentery

Male albino Wistar rats (200–350 g) were killed by a blow to the head and exsanguinated. The abdomen was opened and the ileocolic and colic branches of the superior mesenteric

artery were tied. The portal and superior mesenteric veins were separated from the connecting tissue in the region of the portal vein and a plastic cannula (Portex, size 3FG, external diameter 1.02 mm, internal diameter 0.61 mm) inserted retrogradely into the portal vein and passed into the superior mesenteric vein such that the tip of the cannula was 1.0–1.3 cm distal from the junction of the superior mesenteric and portal veins. The superior mesenteric artery was cannulated, as described before (McGregor, 1965), with an identical cannula and the superior mesenteric vascular bed perfused via the artery for 5 min at 2 ml min⁻¹ with Krebs buffer (at room temperature) containing heparin (100 u ml⁻¹). During this time the effluent from the bed passed out from the venous cannula. At the end of this period the intestine was separated from the mesentery by cutting close to the intestinal border of the mesentery and the preparation supported on a Petri dish while the arteries and veins were perfused independently at 2 ml min⁻¹, with warmed (37.5°C) and gassed (95% O₂:5% CO₂) Krebs buffer containing indomethacin (5 μ M). Changes in perfusion pressures were measured by means of pressure transducers approximately 15 cm from the tips of the arterial and venous cannulae.

Vasoconstrictor responses

After an equilibration period of 45 min vasoconstrictor responses of the two sides of the preparation were tested by

injection of bolus doses (1–15 μ l) of agents into both parts of the circulation, with a time delay between the injections into each side of less than 5 s. Individual drugs were administered at intervals of more than 30 min to minimize desensitization.

Vasodilator responses

In some preparations U46619 (9,11-dideoxy-9 α -methano epoxyprostaglandin F_{2 α} , 0.3–3 μ M) was infused intra-arterially and intravenously to produce an initial peak (24.5 ± 3.5 mmHg, $n = 8$) and then a sustained (17.8 ± 2.5 mmHg, $n = 8$) increase of perfusion pressure in the arterial vasculature that were not significantly different; and a peak (10.5 ± 1 mmHg) and sustained increase (6.3 ± 1 mmHg, $n = 8$) in the perfusion pressure of the venous vasculature, which were significantly different ($P < 0.01$). Thus, the venous vessels did not maintain tone as reliably as the arterial vessels and, although higher concentrations of U46619 did cause larger peak increases in perfusion pressure (data not shown), they did not increase the sustained perfusion pressure. Vasodilatations in response to bolus doses of drugs, administered into both sides of the preparation, were then recorded. Drugs were injected in volumes of 1 μ l to minimize vascular effects due to an injection artefact. In order to discriminate between endothelium-dependent and independent vasodilatations in some preparations gossypol (3 μ M), an inhibitor of EDRF-dependent responses (Förstermann *et al.*, 1986), was infused throughout the experiment.

In all experiments in which the vasodilator responses of the arterial and venous vessels were examined, the arterial portion of the vasculature was perfused throughout at 5 ml min⁻¹. This increased flow rate was found to decrease greatly the spontaneous activity of the arterial vessels which occurred when a contractile agent was infused in preparations perfused at 2 ml min⁻¹.

Intact perfused mesentery and intestine

In a further series of experiments, the pressure responses of the entire perfused mesentery and intestine were recorded to assess the relative contribution of the arterial and venous portions of the mesentery to the responses of the whole vascular bed. The preparation was perfused through the artery with Krebs buffer (2 ml min⁻¹) containing indomethacin (5 μ M) and bovine serum albumin (0.5% w/v) and the effluent allowed to pass to waste out of the venous cannula. In order to avoid the build up of fluid within the lumen of the small intestine, and an increase in the basal perfusion pressure, the intestinal lumen was flushed through for 10 min at 2 ml min⁻¹ with Krebs buffer, at the beginning of the experiment, and holes made with a 19 gauge needle at approx. 1 cm intervals along the length of the intestine. After control responses had been obtained the mesentery was dissected clear of the intestine and the responses of the independently perfused arterial and venous circulations tested.

Materials

The Krebs buffer had the following composition (mM): NaCl 118, KCl 4.7, KH₂PO₄ 1.2, MgSO₄·7H₂O 1.17, CaCl₂·6H₂O 2.5, NaHCO₃ 25 and glucose 5.6. Acetylcholine chloride, adenosine diphosphate di(monocyclohexylammonium) salt (ADP), albumin (bovine fraction V), angiotensin I acetate, angiotensin II acetate, arginine-vasopressin acetate, ascorbic acid sodium salt, bradykinin acetate, gossypol, 5-hydroxytryptamine creatinine sulphate complex, indomethacin, isoprenaline hydrochloride, noradrenaline bitartrate and sodium nitroprusside were purchased from Sigma Chemical Co., Poole, Dorset. Heparin sodium was obtained from Evans Medical Ltd, Greenford, Middlesex. Captopril was obtained from Squibb. U46619 was a generous gift of J. Pike (Upjohn, Kalamazoo, MI, U.S.A.). Endothelin-1 was obtained from Scientific Marketing Associates, London.

All drugs were dissolved in either saline or distilled water except for noradrenaline and 5-hydroxytryptamine, which were dissolved in ascorbic acid (5 mM); indomethacin, which was prepared as a stock solution of 14 mM in NaHCO₃ (5% w/v); and gossypol, which was prepared as a stock solution of 2 mM in ethanol.

Statistics

Results are shown as mean values $\pm$ s.e.mean for n experiments. Student's unpaired t test was used to determine the significance of differences between means and a P value of less than 0.05 was taken as significant.

Results

Perfusion pressure

At a flow of 2 ml min⁻¹, there was a significant difference between the basal perfusion pressures of the arterial (11.5 ± 1 mmHg, $n = 8$) and venous vasculatures (5.5 ± 1 mmHg, $n = 8$). When the arterial portion was perfused at 5 ml min⁻¹ the basal perfusion pressure of the arterial vasculature was significantly raised (17.5 ± 1.5 mmHg, $n = 13$) whereas the venous perfusion pressure was not different from control (4.0 ± 0.5 mmHg, $n = 13$).

The intact perfused mesentery and intestine had a basal perfusion pressure of 22 ± 3.5 mmHg ($n = 7$). When the preparation was separated and the arterial and venous vasculatures perfused independently the arterial and venous basal perfusion pressures were 13 ± 3 mmHg ($n = 7$) and 10 ± 2 mmHg ($n = 7$), respectively.

Intact perfused mesentery and intestine

In the intact perfused mesentery and intestine angiotensin II (30 pmol), arginine vasopressin (30 pmol), noradrenaline (1 nmol) and endothelin-1 (30 pmol) all caused vasoconstrictions ($n = 5$ –6, Figure 1). When the intestine was removed and the arterial and venous portions of the mesentery perfused individually the responses to arginine vasopressin and noradrenaline were associated with the arterial portion of the vasculature and those to angiotensin II with the venous vasculature. Endothelin-1 constricted both parts of the circu-

Figure 1 The role of the arterial and venous vessels in the responses of the intact perfused mesentery. (a) The responses of the whole mesentery. (b) The responses of the separated mesentery, with the responses of the arterial vessels at the top and the venous vessels at the bottom. The vasoconstrictions of the intact mesentery to noradrenaline (NA, 1 nmol) and arginine-vasopressin (AVP, 30 pmol) were matched by the vasoconstrictions of the arterial portion and those to angiotensin II (AII, 30 pmol) by the venous portion. Endothelin-1 (ET-1, 30 pmol) constricted both the arterial and the venous vessels.

Figure 2 Comparison of the contractile responses of the arterial and venous vessels of the rat perfused mesentery. Traces from two experiments, (a) and (b), are shown. The responses of the arterial vessels are shown in the upper panel and those of the venous vessels in the lower panel. KCl ($60 \mu\text{mol}$), noradrenaline (NA, 3 nmol), and 5-hydroxytryptamine (5-HT, 3 nmol) caused principally vasoconstrictions of the arterial circulation whereas U46619 (U4, 300 pmol) principally constricted the venous circulation. Angiotensin II (AII, 100 pmol) caused only constriction of the venous vessels and arginine vasopressin (AVP, 100 pmol) only the arterial vessels. Endothelin-1 (ET-1, 100 pmol) constricted both sides of the circulation but produced a markedly more prolonged effect on the venous than the arterial vessels.

Figure 4 Comparison of the time course of response to constrictors within the arterial and venous portions of the rat mesenteric vascular bed. Arterial (solid columns) and venous responses (hatched columns) are shown. The bars represent the duration of the responses, i.e. the time for the response to return to baseline. Arginine vasopressin (AVP, 0.003 – 0.1 nmol), 5-hydroxytryptamine (5-HT, 0.3 – 10 nmol) and noradrenaline (NA, 0.3 – 10 nmol) were arterioselective vasoconstrictors, but only arginine vasopressin caused significantly longer vasoconstrictions of the arterial than venous vessels. Angiotensin II (AII, 0.003 – 0.1 nmol), U46619 (0.01 – 0.3 nmol) and endothelin-1 (ET-1, 0.003 – 0.1 nmol) all caused significantly more prolonged vasoconstrictions of the venous than arterial vessels. Mean data from 4–5 experiments. Vertical bars show s.e.mean. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$; significant differences between the time course of response of the arterial and venous vessels.

Figure 3 Comparison of constrictors within the arterial and venous portions of the rat mesenteric vascular bed. Arterial responses (solid columns) and venous responses (hatched columns) are shown. Arginine vasopressin (AVP, 0.003 – 0.1 nmol), 5-hydroxytryptamine (5-HT, 0.3 – 10 nmol) and noradrenaline (NA, 0.3 – 10 nmol) were arterioselective vasoconstrictors. Conversely, angiotensin II (AII, 0.003 – 0.1 nmol) and U46619 (0.01 – 0.3 nmol) were selective venoconstrictors. Endothelin-1 (ET-1, 0.003 – 0.1 nmol) constricted both portions of the circulation but the threshold dose for constriction was lower on the venous than the arterial vessels. Mean data from 4–5 experiments. Vertical bars show s.e.mean. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$; significant differences between the responses of the arterial and venous vessels.

lation, although the venous response was maintained for a longer period (Figure 1).

Vasoconstrictor responses

The arterial and venous portions of the mesenteric bed demonstrated distinct profiles of sensitivity to a range of contractile agents. Noradrenaline (0.3 – 10 nmol), 5-hydroxytryptamine (0.3 – 10 nmol) and KCl (1 – $60 \mu\text{mol}$) were significantly more active as constrictors of the arterial portion of the vasculature than the venous portion, whereas U46619 (10 – 300 pmol) was more active as a constrictor of the venous vasculature (Figures 2 and 3). Interestingly, however, the time courses of the responses of the arterial and venous vessels to noradrenaline and 5-hydroxytryptamine were not significantly different, although the vasoconstrictions produced by U46619 were more prolonged on the venous than arterial vessels (Figure 4). Arginine vasopressin (3 – 100 pmol) was found to be a selective constrictor of the arterial vessels and angiotensin II (3 – 100 pmol) a selective venoconstrictor, estimated either as the peak (Figure 3) or duration (Figure 4) of the vasoconstrictor response. Endothelin-1 (3 – 100 pmol) constricted both sides of the vasculature (Figures 2 and 3), but its effect was significantly prolonged on the venous as compared to the arterial portion at all doses tested (Figures 2 and 4).

Figure 5 Bioassay of the exchange of perfusate between the arterial and venous portions of the rat isolated perfused mesentery. The responses of the arterial portion of the circulation are shown in (a) and those of the venous circulation in (b). Three hundred nmol noradrenaline (NA) injected i.v. caused a smaller constriction of the arterial portion of the vasculature than 3 nmol noradrenaline given i.a. and 3 nmol angiotensin II (AII) given i.a. produced a smaller venoconstriction than 0.03 nmol angiotensin II injected i.v.

Role of angiotensin converting enzyme in the responses of the isolated perfused veins

Angiotensin I (0.3 nmol) produced vasoconstrictions of the venous vasculature (14.5 ± 2.3 mmHg, $n = 4$) that were abolished by infusion of captopril ($10 \mu\text{M}$) for 20 min (1 ± 0.5 mmHg, $n = 4$, $P < 0.005$). Vasoconstrictions in response to angiotensin II (0.01 nmol; 6.5 ± 2 mmHg, $n = 4$) were unaffected by captopril (8 ± 1.5 mmHg, $n = 4$).

Independence of the arterial and venous circulations

The exchange of perfusate from one side of the vasculature to the other was estimated, by bioassay, to be less than 1%; 300 nmol noradrenaline injected intravenously caused a vasoconstriction of the arterial portion less than that to 3 nmol noradrenaline injected intra-arterially ($n = 4$, Figure 5). Conversely, 3 nmol angiotensin II given intra-arterially caused a

Figure 7 Comparison of dilators within the arterial and venous vessels of the rat mesentery. Dilatations are expressed as % reduction in induced tone. Responses of arterial vessels (solid columns) and of venous vessels (hatched columns) are shown. Acetylcholine (ACh, 0.01–100 nmol), ADP (0.01–100 nmol) and sodium nitroprusside (NaNP, 0.01–10 nmol) were significantly more active as dilators of the arterial than the venous circulations. Bradykinin (BK, 0.001–1 nmol) produced a poor dose-related dilatation of the arterial vessels but at a dose of 1 nmol was a significantly more potent dilator of the venous than the arterial vessels. Isoprenaline (Iso, 0.001–0.1 nmol) was more active as dilator of venous than arterial vessels. Mean data from 3–9 experiments. Vertical bars show s.e.mean. * $P < 0.05$, ** $P < 0.005$; significant differences between the responses of the arterial and venous vessels.

venoconstriction less than that produced by 0.03 nmol angiotensin II administered intravenously ($n = 4$, Figure 5).

Vasodilator responses

In precontracted preparations, injections of acetylcholine (0.01–100 nmol), bradykinin (0.001–1 nmol), sodium nitroprusside (0.01–10 nmol) or isoprenaline (1–100 pmol) produced dose-related vasodilations of both the arterial and venous sides of the vasculature (Figures 6 and 7). In contrast, ADP (0.01–100 nmol) while producing vasodilations of the arterial

Figure 6 Vasodilator responses of the arterial and venous vessels of the rat mesentery. The responses of the arterial vessels are shown in (a) and those of the venous vessels in (b). Sodium nitroprusside (NaNP, 0.01–10 nmol), acetylcholine (ACh, 0.01–100 nmol) and bradykinin (BK, 0.001–1 nmol) caused dilatations of both circulations. ADP (0.01–100 nmol) caused dilatations of the arterial vessels but principally constrictions of the venous vessels. Isoprenaline (Iso, 0.001–0.1 nmol) caused dilatations of both sides of the circulation but was particularly potent as a venodilator.

Figure 8 The effects of gossypol ($3 \mu\text{M}$) on dilations in the arterial and venous vessels of the rat isolated perfused mesentery. Dilatations are expressed as % of induced tone. (a) Arterial responses and (b) venous responses. Control responses are shown as solid columns and those in the presence of gossypol as hatched columns. Gossypol inhibited dilations to both the arterial and venous vessels to acetylcholine (ACh, 0.01 – 100 nmol), ADP (0.01 – 100 nmol) and bradykinin (BK, 0.001 – 1 nmol). Dilatations to sodium nitroprusside (NaNP, 0.01 – 10 nmol) were not affected. Mean values from 3–9 experiments. Vertical bars show s.e.mean. * $P < 0.05$, ** $P < 0.005$; significant differences between the responses of the arterial and venous vessels.

portion of the bed (Figures 6 and 7) was a poor dilator of the venous portion of the vasculature, where it caused principally vasoconstriction (Figure 6).

Effects of gossypol

In the presence of gossypol ($3 \mu\text{M}$) infusion of U46619 ($0.3 \mu\text{M}$) caused an increase in perfusion pressure of the arterial vasculature ($13 \pm 2 \text{ mmHg}$, $n = 6$) that was not significantly different from control. In contrast, in the venous vasculature U46619 produced a significant increase in perfusion pressure ($3 \pm 0.5 \text{ mmHg}$) in only 3 out of 6 experiments. Relaxations in response to acetylcholine and bradykinin, in both sides of the vasculature, and to ADP, in the arterial vasculature, were inhibited with respect to control, whereas those to sodium nitroprusside were not (Figure 8).

Discussion

This paper describes a new method that allows the simple measurement of the perfusion pressures at constant flow within the arterial and venous circulations of the isolated perfused mesentery of the rat. The results show that there is a clear distinction between the effects on the two sides of the circulation of a number of agents. For instance, angiotensin II constricts only the venous portion of the vascular bed and arginine vasopressin only the arterial portion. ADP, for its part, causes dilatation of the arterial vessels but constriction of the venous ones. Thus, these vasoactive agents demonstrate pronounced arterial-venous selectivity.

The finding that the venous portion of the mesentery is strongly constricted by angiotensin II is supported by an

earlier observation that, *in vivo*, angiotensin II causes an initial increase in the venous return associated with the hepatosplanchnic bed (Emerson, 1966) which is suggestive of venoconstriction in this circulation. Conversely, and as the results presented here suggest, arginine vasopressin does not cause this initial increase (Emerson, 1966). However, as would be expected of such a potent constrictor of the arterial vessels (Bhardwaj & Moore, 1988; Randall *et al.*, 1988), it does cause a sustained increase in the mesenteric perfusion pressure *in vivo* (Dresel & Wallentin, 1966). Endothelin-1 is a constrictor of the arterial vessels of the rat mesenteric vascular bed (de Nucci *et al.*, 1988). The results presented here confirm these findings but also demonstrate that endothelin-1 is an even more potent and prolonged constrictor of the venous portion of the vasculature, as has been found in previous comparisons of arterial and venous vessels (de Nucci *et al.*, 1988). Indeed, even though there was no difference between the peak responses of the arterial and venous vessels to the maximum dose of endothelin-1 used (100 pmol), the duration of the response of the venous vessels was more than twice that of the arterial vessels. This may well explain why endothelin-1 causes such a pronounced and prolonged increase in resistance in the mesentery and intestine *in vivo* (Fortes *et al.*, 1989; Hiley *et al.*, 1989; Walder *et al.*, 1989).

Interestingly, the venous portion of the vasculature did not constrict to noradrenaline, although either stimulation of the sympathetic supply to the mesentery or administration of noradrenaline into this circulation is associated with pronounced and maintained constriction of the mesenteric capacitance vessels (Mellander, 1960; Folkow *et al.*, 1964; Oberg, 1967; Henrich, 1985). This lack of response may reflect the low basal venous perfusion pressure, for the mesenteric capacitance vessels do not constrict well to nerve stimulation when the intraluminal pressure is lower than about 10 mmHg (Oberg, 1967).

As shown previously, acetylcholine (Byfield *et al.*, 1986; Furchgott *et al.*, 1987), ADP (Byfield *et al.*, 1986; Ralevic & Burnstock, 1988) and bradykinin caused endothelium-dependent dilations of the arterial portion of the rat mesentery, as confirmed by their inhibition by gossypol (Forstermann *et al.*, 1986), which inhibits EDRF-mediated vasodilations in this preparation (Bhardwaj & Moore, 1988). The venous circulation also dilated in an endothelium-dependent manner in response to acetylcholine and bradykinin, but primarily constricted in response to ADP. It has been found previously that acetylcholine causes endothelium-dependent relaxation of isolated arteries but principally constriction of isolated veins (De Mey & Vanhoutte, 1982; Vedernikov *et al.*, 1988), whereas *in vivo* it may cause venodilation (Emerson, 1968). The results presented here support those found *in vivo* and show that the principal response of the capacitance vessels to acetylcholine is indeed dilatation. In the organ bath the indirect dilator effects of acetylcholine, mediated by the endothelium, may be over-ridden by constrictor effects, mediated directly by the smooth muscle, for acetylcholine has greater access to the smooth muscle than in a perfused system. As previously found for ATP (De Mey & Vanhoutte, 1982), ADP caused only transient dilations of the venous circulation followed by more prominent vasoconstrictions. Interestingly, bradykinin also causes primarily constriction of isolated mesenteric veins (Toda *et al.*, 1987) and is relatively poor at releasing EDRF from mesenteric arteries (Cherry *et al.*, 1982). Here, in a perfused system, bradykinin was a potent dilator of the capacitance vessels through the release of EDRF and, as previously shown, a relatively poor releaser of EDRF from the arterial vessels. Once again this difference in the activity of bradykinin in venous vessels may be explained by the different experimental systems used. In contrast to the results presented here, bradykinin constricts the venous portion of the mesenteric circulation *in vivo* (Emerson, 1967). However, this can be explained as a secondary effect due to bradykinin-induced constriction of the intestinal smooth muscle. Interestingly, although it is generally

accepted that nitrates have a stronger effect on veins than arteries (Ablad & Mellander, 1963) such a difference in activity was not seen in this preparation. Indeed, as described earlier the veins were particularly sensitive to the dilator effects of isoprenaline (Folkow *et al.*, 1963; De Mey & Vanhoutte, 1982).

The finding that the venous portion of the vasculature converts angiotensin I to angiotensin II demonstrates the presence of active angiotensin converting enzyme in these vessels. As bradykinin is a potent dilator of the capacitance vessels and since it is inactivated by angiotensin converting enzyme, this shows that the venous vessels act to increase the amounts of the venoconstrictor, angiotensin II, while decreasing the levels of the venodilator, bradykinin. Thus, angiotensin converting enzyme inhibitors, such as captopril, will tend to cause a reduction in the tone of these venous vessels by two complementary mechanisms.

Clearly, although this preparation lacks the majority of the resistance vessels of the intact mesentery and intestine (Richardson & Zweifach, 1970; Singbartl & Henrich, 1974; Bohlen & Gore, 1977), it reflects the responses of the intact mesenteric circulation, for there is a direct correlation between systemic arterial pressure and the pressure throughout the microvascular arterial tree (Bohlen & Gore, 1977). Furthermore, although *in vivo* intravenous administration of noradrenaline does not cause constriction of the arterial vessels within the mesentery, but does constrict arterioles within the intestine (Hebert & Marshall, 1985), these former vessels are sensitive to the contractile effects of noradrenaline (McGregor, 1965). This apparent discrepancy is explained by studies that have shown that, within the arterial vessels of the frog mesentery, the maximum constrictor response to noradrenaline is achieved when the tangential stress of these vessels is within an optimum range (Gore, 1972). Thus, *in vivo*, the increase in wall stress of the large arteries, caused by constriction of arterioles further downstream, may prevent any further

decrease in diameter of these vessels. In other words, under these conditions the larger arterial vessels constrict isometrically rather than isotonicity (Hebert & Marshall, 1985). Thus, this preparation will indeed give information about the responses of the entire bed.

Another question is whether or not the responses of the veins have a significant role in the responses of the entire vascular bed. The results presented here suggest that they do, for the responses of the intact mesentery and small intestine to angiotensin II, and in some respects to endothelin-1, were associated with the venous part of the vasculature. Indeed, it has been demonstrated previously that 60–80% of an increase in venous pressure within the mesenteric circulation is directly reflected as an increase in arterial resistance (Selkurt & Johnson, 1958; Folkow *et al.*, 1963; Richardson & Zweifach, 1970), possibly because the venous vessels do not contain many venular valve-like structures (Gaetgens & Uekermann, 1971).

Finally, what is the relevance of this to the whole circulation? As the mesenteric vascular bed may be the destination for 10% of the cardiac output, obviously changes in this can have important effects on the whole cardiovascular system. For instance, removal of the hepatosplanchnic vasculature *in vivo* abolishes both the increase in venous return caused by acetylcholine (Emerson, 1968) and the initial increase in venous return caused by angiotensin II (Emerson, 1966). It is concluded that this preparation can supply useful information about the behaviour of the mesenteric circulation *in vivo* as well as providing a simple system in which many of the difficult to study responses of the venous portion of the vasculature may be examined.

I am indebted to Professor Sir John Vane and Dr Pedro D'Orléans-Juste for helpful discussion and editorial assistance.

The William Harvey Research Institute is supported by a grant from Glaxo Group Research Limited.

References

- ABLADE, B. & MELLANDER, S. (1963). Comparative effects of hydralazine, sodium nitrite and acetylcholine on resistance and capacitance blood vessels and capillary filtration in skeletal muscles in the cat. *Acta Physiol. Scand.*, **58**, 319–329.
- BHARDWAJ, R. & MOORE, P.K. (1988). Endothelium-derived relaxing factor and the effects of acetylcholine and histamine on resistance blood vessels. *Br. J. Pharmacol.*, **95**, 835–843.
- BOHLEN, H.G. & GORE, R.W. (1977). Comparison of microvascular pressures and diameters in the innervated and denervated rat intestine. *Microvasc. Res.*, **14**, 251–264.
- BYFIELD, R.A., SWAYNE, G.T.G. & WARNER, T.D. (1986). A method for the study of endothelial derived relaxing factor (EDRF) in the isolated perfused rat mesentery. *Br. J. Pharmacol.*, **88**, 438P.
- CHERRY, P.D., FURCHGOTT, R.F., ZAWADZKI, J.V. & JOTHIANADAN, D. (1982). Role of endothelial cells in relaxation of isolated arteries by bradykinin. *Proc. Natl. Acad. Sci. U.S.A.*, **79**, 2106–2110.
- DE MEY, J.G. & VANHOUTTE, P.M. (1982). Heterogenous behaviour of the canine arterial and venous wall. *Circ. Res.*, **51**, 439–447.
- DRESEL, P. & WALLENTIN, I. (1966). Effects of sympathetic vasoconstrictor fibres, noradrenaline and vasopressin on the intestinal vascular resistance during constant blood flow or blood pressure. *Acta Physiol. Scand.*, **66**, 427–436.
- EMERSON, T.E. JR. (1966). Effects of angiotensin, epinephrine, norepinephrine, and vasopressin on venous return. *Am. J. Physiol.*, **210**, 933–942.
- EMERSON, T.E. JR. (1967). Changes of venous return and other hemodynamic parameters during bradykinin infusion. *Am. J. Physiol.*, **212**, 1455–1460.
- EMERSON, T.E. JR. (1968). Effects of acetylcholine, histamine, and serotonin infusion on venous return in dogs. *Am. J. Physiol.*, **215**, 41–48.
- FOLKOW, B., LUNDGREN, O. & WALLENTIN, I. (1963). Studies on the relationship between flow resistance, capillary filtration coefficient and regional blood volume in the intestine of the cat. *Acta Physiol. Scand.*, **57**, 270–273.
- FOLKOW, B. & MELLANDER, S. (1964). Veins and venous tone. *Am. Heart. J.*, **68**, 397–408.
- FOLKOW, B., LEWIS, D.H., LUNDGREN, O., MELLANDER, S. & WALLENTIN, I. (1964). The effect of graded vasoconstrictor fibre stimulation on the intestinal resistance and capacitance vessels. *Acta Physiol. Scand.*, **61**, 445–457.
- FÖRSTERMANN, U., GOPPELT-STRÜBE, M., FRÖLICH, J.C. & BUSSE, R. (1986). Inhibitors of acyl-coenzyme A: lysocleithin acyltransferase activate the production of endothelium-derived relaxing factor. *J. Pharmacol. Exp. Ther.*, **238**, 352–359.
- FORTES, Z.B., DE NUCCI, G. & GARCIA-LEME, J. (1989). Effect of endothelin on arterioles and venules *in vivo*. *J. Cardiovasc. Pharmacol.*, **13** (suppl. 5), S200–S201.
- FURCHGOTT, R.F., CARVALHO, M.H., KHAN, M.T. & HATSUNAGA, K. (1987). Evidence for endothelium-dependent vasodilatation of resistance blood vessels by acetylcholine. *Blood Vessels*, **24**, 145–149.
- GAEHTGENS, P. & UEKERMANN, U. (1971). The distensibility of mesenteric venous microvessels. *Pflügers Arch.*, **330**, 206–216.
- GORE, R.W. (1972). Wall stress: a determinant of regional differences in response of frog microvessels to norepinephrine. *Am. J. Physiol.*, **222**, 82–91.
- HEBERT, M.T. & MARSHALL, J.M. (1985). Direct observations of responses of mesenteric microcirculation of the rat to circulating noradrenaline. *J. Physiol.*, **368**, 393–407.
- HENRICH, H.A. (1985). Precapillary control of the splanchnic vascular bed. *J. Cardiovasc. Pharmacol.*, **7** (suppl. 3), S73–S79.
- HILEY, C.R., DOUGLAS, S.A. & RANDALL, M.D. (1989). Pressor effects of endothelin-1 and some analogs in the perfused superior mesenteric arterial bed of the rat. *J. Cardiovasc. Pharmacol.*, **13** (suppl. 5), S197–S199.
- MELLANDER, S. (1960). Comparative studies on the adrenergic neurohumoral control of resistance and capacitance blood vessels in the cat. *Acta Physiol. Scand.*, **50** (suppl. 176), 1–86.
- MCGREGOR, D.D. (1965). The effect of sympathetic nerve stimulation on vasoconstrictor responses in perfused mesenteric blood vessels of the rat. *J. Physiol.*, **177**, 21–30.
- DE NUCCI, G., THOMAS, G.R., D'ORLEANS-JUSTE, P., ANTUNES, E., WALDER, C., WARNER, T.D. & VANE, J.R. (1988). The pressor

- effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and EDRF. *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 9797-9800.
- OBERG, B. (1967). The relationship between active constriction and passive recoil of the veins at various distending pressures. *Acta Physiol. Scand.*, **71**, 233-247.
- RALEVIC, V. & BURNSTOCK, G. (1988). Actions mediated by P₂-purinoceptors in the isolated perfused mesenteric bed of the rat. *Br. J. Pharmacol.*, **95**, 637-645.
- RANDALL, M.D., KAY, A.O. & HILEY, C.R. (1988). Endothelium-dependent modulation of the pressor activity of arginine-vasopressin in the isolated superior mesenteric arterial bed of the rat. *Br. J. Pharmacol.*, **95**, 646-652.
- RICHARDSON, D.R. & ZWEIFACH, B.W. (1970). Pressure relationships in the macro- and micro-circulation of the mesentery. *Microvasc. Res.*, **2**, 474-488.
- SELKURT, E.E. & JOHNSON, P.C. (1958). Effect of acute elevation of portal venous pressure on mesenteric blood volume, interstitial fluid volume and haemodynamics. *Circ. Res.*, **6**, 592-599.
- SINGBARTL, G. & HENRICH, H. (1974). Adrenergic-induced vascular adjustments—initial and escape reactions. *Pflügers Arch.*, **346**, 13-24.
- TODA, N., BIAN, K., AKIBA, T. & OKAMURA, T. (1987). Heterogeneity in mechanisms of bradykinin action in canine isolated vessels. *Eur. J. Pharmacol.*, **135**, 321-329.
- VEDERNIKOV, Y.P., GRASER, T., TIEDT, N. & VIKHERT, A.M. (1988). Heterogeneity of the response of venous smooth muscle to arterial endothelium-derived relaxing factor (EDRF) in respect of the role of nitric oxide. *Basic Res. Cardiol.*, **83**, 122-127.
- WALDER, C.E., THOMAS, G.R., THIEMERMANN, C. & VANE, J.R. (1989). The hemodynamic effects of endothelin-1 in the pithed rat. *J. Cardiovasc. Pharmacol.*, **13** (suppl. 5), S93-S97.

(Received August 14, 1989

Revised September 27, 1989

Accepted October 3, 1989)