Br. J. Pharmacol. (1993), **109**, 195–199

Characterization of the increase in vascular permeability induced by vascular permeability factor *in vivo*

[1]P.D. Collins, *D.T. Connolly & T.J. Williams

Department of Applied Pharmacology, National Heart and Lung Institute, Dovehouse Street, London SW3 6LY and *Monsanto Company, St Louis, Missouri, U.S.A.

1 Vascular permeability factor (VPF) is a protein secreted from a variety of human and rodent tumour and normal tissue cells. In addition to mediating angiogenesis and endothelial cell growth, VPF has been reported to be a potent mediator of increased microvascular permeability *in vivo*. In this study we have investigated these permeability changes *in vivo* using a quantitative model of local plasma leakage in rabbit skin.

2 Our results reveal that VPF is a potent mediator of plasma leakage which, in the rabbit, depends on a synergistic interaction with arteriolar vasodilators such as prostaglandin E_2. The requirement for an exogenous vasodilator further suggests that VPF does not act to increase blood flow in this model.

3 We show that this response does not require the presence of circulating neutrophils and in this respect is similar to direct-action permeability increasing mediators such as histamine and bradykinin. Similarly, the time course of plasma leakage induced by VPF resembles that of direct-action mediators, where the greatest response occurs over the first 30 min. In contrast, the neutrophil-dependent plasma leakage induced by the active component of zymosan-activated plasma, $C5a_{des\ arg}$, was maintained at a similar level over 2.5 h.

4 Further, using mediator antagonists and enzyme inhibitors we demonstrate that the mechanism of action of VPF is not via activation of histamine, kinin, or platelet-activating factor pathways.

Keywords: Vascular permeability factor; permeability; vascular endothelial growth factor

Introduction

The growth of tumours is often associated with plasma extravasation from 'leaky' tumour vasculature (O'Connor & Bale, 1984; Brown *et al.*, 1988). Investigation of this phenomenon has revealed that several animal and human tumour cell lines secrete a vascular permeability factor, VPF (Senger *et al.*, 1983; 1986; Connolly *et al.*, 1989b; Clauss *et al.*, 1990). VPF is a 34–42 kDa glycoprotein which has an identical amino acid sequence to vascular endothelial growth factor (VEGF), a product of bovine folliculostellate cells (Ferrara & Henzel, 1989; Leung *et al.*, 1989). In addition to its effects on vascular permeability, VPF is a mitogen for endothelial cells (Connolly *et al.*, 1989a,b) and is able to induce angiogenesis *in vivo* (Connolly *et al.*, 1989a). The mechanisms by which VPF exerts its effects on microvascular permeability are unknown; however, recent *in vitro* studies have demonstrated an interaction between VPF and endothelial cells via specific binding sites (Connolly *et al.*, 1989a; Vaisman *et al.*, 1990; Brock *et al.*, 1991; Olander *et al.*, 1991). *In vivo*, increases in permeability induced by intradermal VPF have been visualized by the local accumulation of intravenously injected Evans blue dye (Senger *et al.*, 1983; Connolly *et al.*, 1989a,b; Keck *et al.*, 1989). In these studies recombinant human and guinea-pig VPF was reported to be active at a concentration of 1–3 nmol making it 5,000 times more potent than histamine (Connolly *et al.*, 1989b). At present, however, the biological profile of VPF as a mediator of increased microvascular permeability has not been quantified nor has its possible interaction with inflammatory cells, such as neutrophils, been investigated.

In this study, therefore, we have carried out detailed experiments to investigate possible mechanisms of increased microvascular permeability induced by VPF *in vivo*. The role of circulating neutrophils and the synergy between VPF and vasodilators is also addressed.

[1] Author for correspondence.

Methods

Human recombinant vascular permeability factor

The 165 amino acid-containing isoform of recombinant human VPF (Leung *et al.*, 1989) was used in these studies. This protein was prepared from the serum-free conditioned medium of baby hamster kidney (BHK) cells which had been stably transfected with a cDNA for VPF. High level expression of VPF was controlled by the herpes virus immediate early promoter in conjunction with co-expression of the herpes virus transactivator, VP-16 (Paul Hippenmeyer and Maureen Highkin, unpublished observations). The VPF protein was purified to homogeneity by cation exchange chromatography and reverse-phase chromatography. Purified VPF was prepared for injection by dilution to the appropriate concentration in saline containing 0.1 mg ml^{-1} BSA.

Rabbit skin bioassay

The pro-inflammatory properties of VPF were investigated by use of an established model in the rabbit to determine changes in microvascular permeability (Wedmore & Williams, 1981). Male rabbits (2.5–3.0 kg) were anaesthetized (Sagatal; sodium pentobarbitone, 30 mg kg^{-1}, i.v.) and the dorsal fur removed. Agents under test were injected intradermally into the dorsal skin in 6 replicate sites (100 µl/skin site) according to a balanced site injection plan. [^{125}I]-labelled human serum albumin ([^{125}I]-HSA; 2.5 µCi/animal) and Evans blue dye (2.5% w/v in sterile saline, 0.5 ml kg^{-1}) were injected intravenously as quantitative and visual markers respectively of plasma protein leakage into the sites. At the end of the measurement period, blood was collected by cardiac puncture and the animal killed with an overdose of anaesthetic (Expiral; sodium pentobarbitone, 200 mg kg^{-1}, i.v.). The dorsal skin was removed and excess tissue and blood separated. Skin sites (17 mm diameter) were punched out and radioactivity measured in a multiwell gamma counter. By comparison with the radioactivity associated with 1 ml of plasma, the plasma leakage into the site was calculated as µl plasma leakage/skin site.

Bioassay protocols

Synergy between vascular permeability factor and the vasodilator prostaglandin E_2 In these experiments [^{125}I]-HSA and Evans blue dye was given 10 min prior to the intradermal injections. VPF, formyl-methionyl-leucyl-phenylalanine (FMLP), bradykinin (BK), and rabbit zymosan-activated plasma (ZAP; a source of $C5a_{des\ arg}$) were injected intradermally at various concentrations either alone or in the presence of prostaglandin E_2 (PGE_2; 3×10^{-10} mol 100 µl/site). The response was measured over a 30 min period.

Neutrophil-dependency of the inflammatory response induced by vascular permeability factor Rabbits were treated 3 days before vascular permeability studies with a single injection of mustine hydrochloride (1 mg kg^{-1}; 1.75 ml kg^{-1}, i.v.) to deplete circulating neutrophils by greater than 98%. On day four [^{125}I]-HSA and Evans blue dye was given 10 min before intradermal injections. VPF, BK, histamine (Hist), FMLP, human recombinant C5a (HrC5a), and rabbit ZAP were each co-injected intradermally with PGE_2 and the response measured over 30 min.

Time course of the vascular permeability factor-induced inflammatory response

In these experiments the kinetics of plasma protein leakage induced by VPF, ZAP, and BK were investigated. VPF, BK, and rabbit ZAP were injected intradermally at various times (−240, −120, −60, −30, 0 min) prior to superimposed intradermal injections of PGE_2 at time zero. Intravenous administration of [^{125}I]-HSA and Evans blue dye was carried out at −2 min. The inflammatory response was then measured over a 30 min period to give the rate of leakage over time windows of 0–30, 30–60, 60–90, 120–150, and 240–270 min.

Effect of mediator antagonists, enzyme inhibitors and anti-vascular permeability factor antibodies on plasma leakage

Radiolabel and blue dye were administered 10 min before intradermal injection of VPF together with PGE_2 and in the presence or absence of either a platelet-activating factor (PAF) receptor antagonist WEB 2086, a bradykinin receptor antagonist Hoe 140, a histamine receptor antagonist, mepyramine, an inhibitor of kinin formation, trasylol, or a specific guinea-pig anti-human VPF antibody. These antagonists and inhibitors were also co-injected with their respective agonists, PAF, bradykinin, histamine, kallikrein, and human VPF (at doses selected to give sub-maximal responses) to demonstrate inhibition of the plasma leakage induced by these agents. VPF is a cationic protein and it has previously been demonstrated that highly charged peptides can induce plasma leakage (Needham *et al.*, 1988). We therefore investigated this possibility by co-injecting VPF with heparin (heparin was also co-injected with the polycation poly-L-lysine to demonstrate the inhibitory activity of heparin). The response was measured over 30 min.

Materials

[^{125}I]-human serum albumin ([^{125}I]-HSA, 50 µCi ml^{-1} in sterile saline) was from Amersham International plc, Amersham, Bucks. Bovine serum albumin (BSA, less than 0.1 ng endotoxin per mg protein), bradykinin (BK) histamine acid phosphate (Hist), poly-L-lysine (mol wt. 305,700–370,500), kallikrein, and prostaglandin E_2 (PGE_2) were from Sigma Chemical Company, Poole, Dorset. Mepyramine was from Glaxo Ltd, Ware, Hertfordshire. Trasylol was from Bayer UK Ltd, Newbury, Berkshire. Heparin was from CP Pharmaceuticals Ltd, Wrexham, Clwyd. Evans blue dye was from Merck Ltd, Dagenham, Essex. Hoe 140 (D-Arg^0-Hyp^3-Thi^5-D-Tic^7-Oic^8-bradykinin) was a generous gift from Dr K.J. Wirth, Hoeschst AG, Frankfurt, Germany. WEB 2086 (3-[4-(2-chlorphenyl)-9-methyl-6H-thieno [3,2-f] [1,-2,4]-triazolo-[4,3,a][1,4]-diazepin-[2-yl]-1-(4-morpholinyl)-1-propanone) was a generous gift from Boehringer-Ingelheim KG, Ingelheim am Rhein, Frankfurt, Germany.

Statistical analysis

Statistical significance was assessed by two-way analysis of variance (ANOVA). Normality of experimental data was tested by the Shapiro-Francia test and when necessary (significance of non-normality $P<0.10$), as detailed in the figure legends, $\log_{10}$-transformed data were analysed. A P value of <0.05 was considered significant, however, in all cases significant differences were at a level <0.01 as indicated by an asterisk in the relevant figures.

Results

Synergy between vascular permeability factor and prostaglandin E_2

Figure 1 demonstrates that when injected into rabbit skin, VPF alone induces little microvascular plasma protein leakage. A similar lack of a response can be seen when the inflammatory mediators BK, FMLP, and rabbit ZAP are injected alone. However, when VPF is co-administered with a vasodilator dose of PGE_2 a marked synergy results leading to a significant dose-dependent plasma leakage into the tissue. Comparison with BK, FMLP, and rabbit ZAP co-injected with PGE_2 indicate that VPF is the most active mediator in this model. Subsequent experiments were carried out with all agonists in the presence of PGE_2.

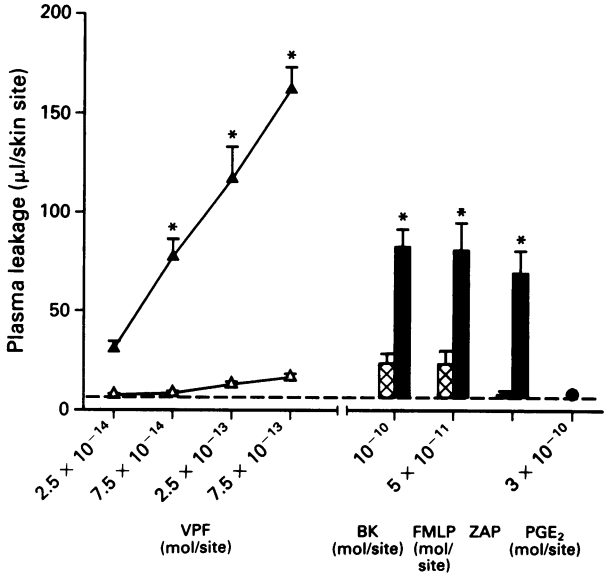

Figure 1 The synergistic interaction between vascular permeability factor (VPF) and prostaglandin E_2 (PGE_2); comparison with other mediators that increase microvascular permeability. VPF, bradykinin (BK), formyl-methionyl-leucyl-phenylalanine (FMLP), and zymosan-activated plasma (ZAP) were injected intradermally in the presence (▲, ■) and absence (△, ▩) of PGE_2 and plasma leakage measured over 30 min. The response to PGE_2 alone (●) and the basal response to intradermal saline (------) is also shown. Results are expressed as the mean plasma leakage ± s.e.mean from 5 separate experiments. Statistical analysis was carried out on untransformed data and * indicates a significant difference from the response to injection of saline alone ($P<0.01$).

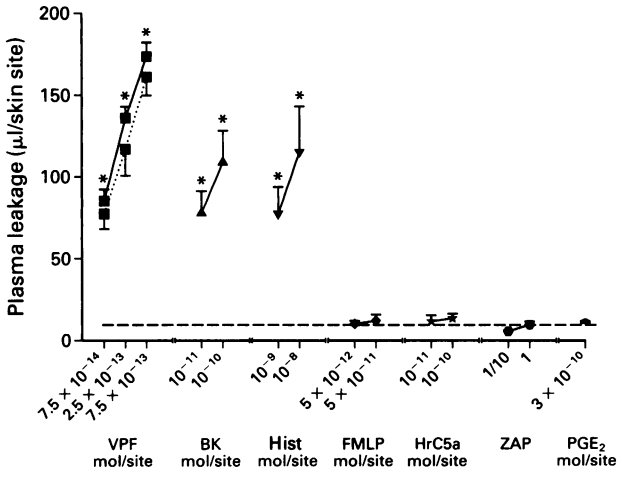

Figure 2 The effect of systemic neutrophil depletion on plasma leakage induced by vascular permeability factor (VPF). VPF (■), BK (▲), Hist (▼), FMLP (◆), HrC5a (★), and ZAP (● undiluted, 1; and diluted 10 fold in saline, 1/10) were injected intradermally in the presence of PGE_2 in animals depleted of circulating neutrophils. Hist = histamine; other abbreviations, see legend to Figure 1. Plasma leakage was measured over 30 min. For comparison the response to VPF + PGE_2 in normal animals is also shown (■····■) together with the response to PGE_2 alone (●) and saline (-----). The results are expressed as the mean plasma leakage/skin site ± s.e.mean from 4 separate experiments. Statistical analysis was carried out on $\log_{10}$-transformed data and * indicates a significant difference from the response to injection of PGE_2 alone ($P<0.01$).

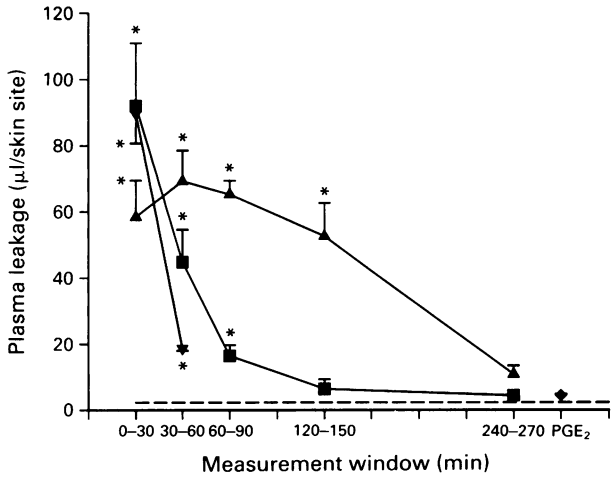

Figure 3 Kinetics of the plasma leakage induced by vascular permeability factor (VPF); comparison with rabbit zymosan-activated plasma (ZAP) and bradykinin (BK). VPF (■, 7.5×10^{-14} mol/site), ZAP (▲) and BK (▼, 5×10^{-10} mol/site) were injected intradermally at various times prior to a super-imposed injection of prostaglandin E_2 (PGE_2, 3×10^{-10} mol/site) at the beginning of the measurement period. Plasma leakage was measured over 30 min. The results are expressed as the mean plasma leakage/skin site ± s.e.mean from 5 separate experiments. The response to PGE_2 alone (◆) and saline (-----) is also shown. Statistical analysis was carried out on $\log_{10}$-transformed data and * indicates a significant difference from the response to injection of PGE_2 alone ($P<0.01$).

Neutrophil-dependency of plasma leakage induced by vascular permeability factor

The results in Figure 2 indicate that, despite depletion of circulating neutrophils, the plasma leakage in response to VPF is unaffected when compared to the response in normal animals. Similarly the increased permeability induced by the direct-action mediators BK and histamine is not inhibited. In contrast the chemoattractant mediators FMLP and rabbit ZAP, whilst active in normal animals (Figure 1), do not cause plasma leakage in these neutrophil-depleted rabbits (Figure 2).

Time course of the inflammatory response to vascular permeability factor

The time course of action of VPF as a mediator of increased microvascular permeability was compared with that of bradykinin, and the chemoattractant component of rabbit ZAP, C5a. Figure 3 shows that, like bradykinin, VPF induces a rapid plasma leakage which is maximal over the first 30 min. The VPF response then progressively declines so that at 2 h no response is observed. Comparison of the response to BK and VPF over 30–60 min indicates that BK has a shorter duration of action than VPF. In contrast, the leakage obtained in response to C5a has a rapid onset that is maintained for at least 2 h, returning to baseline by 4 h.

The effect of antagonists to bradykinin, PAF, and histamine on vascular permeability factor-induced plasma leakage

Co-injection of bradykinin, PAF, or histamine and their respective receptor antagonists (Hoe 140, WEB 2086, and mepyramine) inhibited the plasma leakage observed to the mediators alone (Figures 4, 5). In contrast the leakage induced by VPF was unaffected in the presence of the antagonists suggesting that none of these mediators or their receptors is involved in the plasma leakage response to VPF.

The effect of trasylol, heparin, and anti-human vascular permeability factor antibodies on the plasma leakage induced by VPF

The results displayed in Figure 5 demonstrate that trasylol, an inhibitor of kinin formation, totally inhibited the plasma leakage induced by kallikrein. In contrast, trasylol was with-

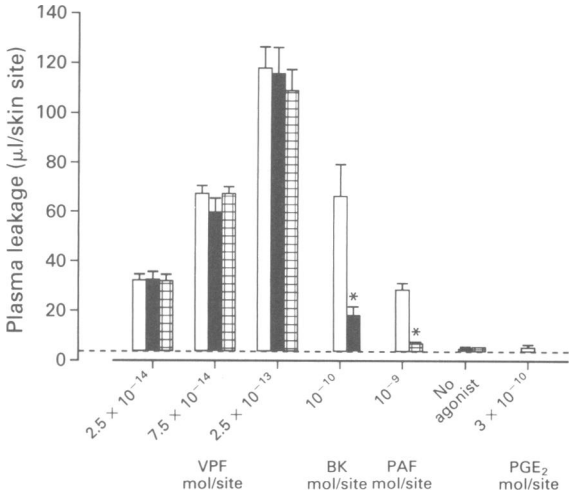

Figure 4 Effect of Hoe 140 and WEB 2086 on plasma leakage induced by vascular permeability factor (VPF). VPF was injected intradermally together with prostaglandin E_2 (PGE_2) alone (□) or in the presence of either the bradykinin antagonist Hoe 140 (■, 10^{-9} mol/site) or the platelet-activating factor (PAF) antagonist WEB 2086 (▦, 10^{-7}mol/site). Bradykinin and PAF were injected intradermally with PGE_2 in the presence (■, ▦) and absence (□) of their respective antagonists. Plasma leakage was measured over 30 min. The results are expressed as the mean plasma leakage/skin site ± s.e.mean from 3 separate experiments. The response to PGE_2 alone (□) and saline (-----) is also shown. Statistical analysis was carried out on untransformed data and * indicates a significant difference between the response to injection of agonist alone and agonist + antagonist ($P<0.01$).

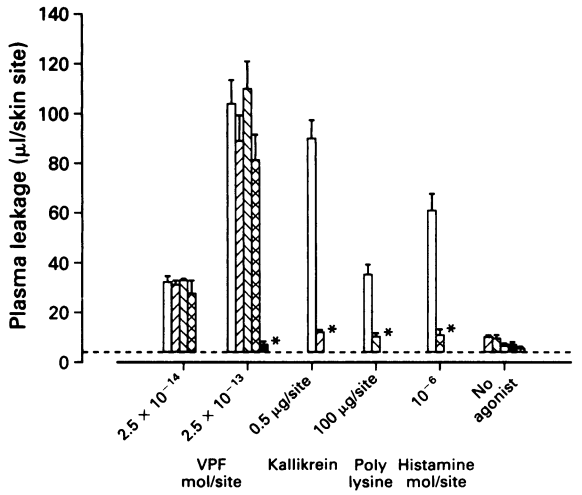

Figure 5 The effect of mepyramine, heparin, trasylol and anti-vascular permeability factor (VPF) antibody on plasma leakage induced by VPF. VPF was injected together with prostaglandin E_2 (PGE_2, 3×10^{-10} mol/site) alone (□) or in the presence of mepyramine (3×10^{-9} mol/site; ⊠), heparin (100 u/site; ▨), trasylol (100 µg/site; ▨), or an anti-VPF antibody (1/3000 dilution in saline; ■). Histamine, poly-L-lysine and kallikrein were injected with PGE_2 in the absence (□) or presence (⊠, ▨, ▨) of their respective antagonists or inhibitors. Plasma leakage was measured over 30 min. The response to PGE_2 alone (□) and saline (-----) is also shown. Results are expressed as the mean plasma leakage/skin site ± s.e.mean from 3–5 separate experiments. Statistical analysis was carried out on untransformed data and * indicates a significant difference between the response to the agonist alone and the agonist + appropriate antagonist ($P < 0.01$).

out effect on the response induced by VPF indicating that VPF does not function via the *in vivo* generation of kinins. Similarly heparin, whilst abolishing the response obtained to the poly-cation poly-L-lysine (average mol wt. = 341,000), had no inhibitory action on the response to VPF. This would indicate that the cationic nature of VPF is not responsible for the plasma leakage observed. The lack of effect of the inhibitors and antagonists was in marked contrast to the total inhibition of VPF-induced plasma leakage following co-injection with an anti-human VPF antibody (1/3000 dilution). In other studies this antibody did not inhibit the plasma leakage induced by histamine, bradykinin, or C5a at a 1/300 dilution (data not shown).

Discussion

Mediators of increased microvascular permeability have been classically divided into two distinct groups (Wedmore & Williams, 1981). This separation is based on the dependency of the response on the interaction between circulating neutrophils and the local post-capillary venular endothelium. Thus, chemoattractant mediators such as C5a, FMLP, and interleukin-8 form one group, whilst the direct-action mediators exemplified by bradykinin and histamine comprise the second group. Other early studies have demonstrated that the increased microvascular permeability induced by both neutrophil-dependent and direct-action mediators is potentiated via a synergistic interaction with agents that produce a local pre-capillary arteriolar vasodilatation. These vasodilators include prostaglandins (PGE_1, PGE_2, PGI_2) (Williams & Morley, 1973; Williams & Peck, 1977; Wedmore & Williams, 1981; Rampart & Williams, 1986) and neuropeptides (calcitonin gene-related peptide, vasoactive intestinal polypeptide) (Williams, 1982; Brain *et al.*, 1985; Brain & Williams, 1985). In this study we have demonstrated that human rVPF can be classed as a direct-action mediator since it induces plasma leakage in the absence of circulating neutrophils. Further evidence for this classification comes from studying the kinetics of the response. Unlike the neutrophil-dependent plasma leakage induced by $C5a_{des\ arg}$ (the active component of ZAP) which is similar over the first 2.5 h, the response to VPF is maximal in the first 30 min, decreasing to basal levels by 2 h. In this respect the profile of the response is similar to that of the direct-action mediator bradykinin which in the time course study also produces maximal plasma leakage over the first 30 min.

In addition, increased microvascular permeability in rabbit skin induced by VPF is entirely dependent on the presence of a vasodilator, in this case exogenous PGE_2. This would suggest that VPF itself does not increase local blood flow in this model, nor does it appear to induce the endogenous production of vasodilators from the local tissue. In contrast, induction of local plasma leakage in the guinea-pig skin does not require the presence of an exogenous vasodilator (Williams & Piper, 1980) due to higher basal blood flow. In this species it has been demonstrated that plasma leakage in response to VPF occurs at a similar concentration (2×10^{-13} mol/site) in the absence of any exogenous vasodilator (Senger *et al.*, 1990). In rabbit skin, at a VPF dose of 7.5×10^{-14} mol/site, a plasma leakage response was induced equivalent to that elicited by the direct-action mediator bradykinin and the neutrophil-dependent mediator FMLP at doses 1300 and 700 times greater respectively.

As part of the investigations into the mechanism of action of VPF the role of other endogenous mediators was studied. One possibility was that VPF caused the release of histamine, kinins, or PAF, or alternatively that VPF acted as an agonist at their receptors. Using specific antagonists we demonstrated almost complete inhibition of plasma leakage induced by sub-maximal doses of histamine, kallikrein, and PAF. There was, however, no effect on the response elicited by VPF, suggesting that neither these mediators nor their receptors are involved in the action of VPF. This would imply the presence of distinct binding sites for VPF *in vivo*, as has been demonstrated *in vitro* on capillary and large vessel endothelial cells from bovine and human tissues (Connolly *et al.*, 1989a; Vaisman *et al.*, 1990; Olander *et al.*, 1991; Gitay-Goren *et al.*, 1992). Indeed, activation of endothelial cells by VPF has been demonstrated by Brock *et al.* (1991) who showed that VPF stimulates a delayed and transient rise in $[Ca^{2+}]_i$, inositol triphosphate formation and von Willebrand factor release.

In addition to demonstrating that the actions of VPF did not appear to be associated with its cationic nature, we observed that co-injection of VPF with heparin did not modulate the leakage response. Gitay-Goren *et al.* (1992) found enhanced binding of VPF to endothelial cells in the presence of heparin and an inhibition of binding following treatment with heparinase suggesting a dependence on heparin for VPF binding. In our study it is possible that the lack of effect of heparin on the response is due to the presence of endogenous heparin associated with the VPF binding site in our *in vivo* model.

The presence of specific receptors for VPF on endothelial cells may suggest that this protein is released from non-tumour cells. It has recently been reported by Berse *et al.* (1992) using *in situ* hybridization techniques in guinea-pig tissue that in addition to secretion of VPF from tumour cells, VPF is expressed in epithelial cells within the pulmonary alveolar wall, the kidney glomerular loop and the outer cortex of the adrenal gland. In the heart, expression is associated with cardiac myocytes. VPF expression has also been detected in elicited guinea-pig peritoneal macrophages but not resident cells implying that activation of the cell is required for VPF generation.

The potency of VPF and the distribution of cells able to secrete this protein make it a candidate as a homeostatic regulator of microvascular permeability under physiological conditions. The release of VPF from activated macrophages may indicate an additional role as a component of the

inflammatory response, forming part of the host defence function of these cells.

Further studies are necessary to determine whether VPF has an important endogenous function and if the association between VPF and tumour cell growth represents a target for therapeutic intervention.

P.D.C. is supported by the Wellcome Trust and T.J.W. is supported by the National Asthma Campaign.

References

BERSE, B., BROWN, L.F., VAN DE WATER, L., DVORAK, H.F. & SENGER, D.R. (1992). Vascular permeability factor (vascular endothelial growth factor) gene is expressed differentially in normal tissues, macrophages, and tumors. *Mol. Biol. Cell*, **3**, 211–220.

BRAIN, S.D. & WILLIAMS, T.J. (1985). Inflammatory oedema induced by synergism between calcitonin gene-related peptide (CGRP) and mediators of increased vascular permeability. *Br. J. Pharmacol.*, **86**, 855–860.

BRAIN, S.D., WILLIAMS, T.J., TIPPINS, J.R., MORRIS, H.R. & MACINTYRE, I. (1985). Calcitonin gene-related peptide (CGRP) is a potent vasodilator. *Nature*, **313**, 54–56.

BROCK, T.A., DVORAK, H.F. & SENGER, D.R. (1991). Tumor-secreted vascular permeability factor increases cytosolic Ca^{2+} and von Willebrand factor release in human endothelial cells. *Am. J. Pathol.*, **138**, 213–221.

BROWN, L.F., ASCH, B., HARVEY, V.S., BUCHINSKI, B. & DVORAK, H.F. (1988). Fibrinogen influx and accumulation of cross-linked fibrin in mouse carcinomas. *Cancer Res.*, **48**, 1920–1925.

CLAUSS, N., GERLACH, M., GERLACH, H., BRETT, J., WANG, F., FAMILLETTI, P.C., PAN, Y.-C.E., OLANDER, J.V., CONNOLLY, D.T. & STERN, D. (1990). Vascular permeability factor: a tumor-derived polypeptide that induces endothelial cell and monocyte procoagulant activity, and promotes monocyte migration. *J. Exp. Med.*, **172**, 1535–1545.

CONNOLLY, D.T., HEUVELMAN, D.M., NELSON, R., OLANDER, J.V., EPPLEY, B.L., DELFINO, J.J., SIEGEL, N.R., LEIMGRUBER, R.M. & FEDER, J. (1989a). Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis. *J. Clin. Invest.*, **84**, 1470–1478.

CONNOLLY, D.T., OLANDER, J.V., HEUVELMAN, D., NELSON, R., MONSELL, R., SIEGEL, N., HAYMORE, B.L., LEIMGRUBER, R. & FEDER, J. (1989b). Human vascular permeability factor. Isolation from U937 cells. *J. Biol. Chem.*, **264**, 20017–20024.

FERRARA, N. & HENZEL, W.J. (1989). Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. *Biochem. Biophys. Res. Commun.*, **161**, 851–858.

GITAY-GOREN, H., SOKER, S., VLODAVSKY, I. & NEUFELD, G. (1992). The binding of vascular endothelial growth factor to its receptors is dependent on cell surface-associated heparin-like molecules. *J. Biol. Chem.*, **267**, 6093–6098.

KECK, P.J., HAUSER, S.D., KRIVI, G., SANZO, K., WARREN, T., FEDER, J. & CONNOLLY, F.T. (1989). Vascular permeability factor, an endothelial cell mitogen related to PDGF. *Science*, **246**, 1309–1312.

LEUNG, D.W., CACHLANES, G., KUANG, W.-J., GOEDDEL, D.V. & FERRARA, N. (1989). Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science*, **246**, 1306–1312.

NEEDHAM, L., HELLEWELL, P.G., WILLIAMS, T.J. & GORDON, J.L. (1988). Endothelial functional responses and increased vascular permeability induced by polycations. *Lab. Invest.*, **59**, 538–548.

O'CONNOR, S.W. & BALE, W.F. (1984). Accessibility of circulating immunoglobulin G to the extravascular compartment of solid rat tumors. *Cancer Res.*, **44**, 3719–3723.

OLANDER, J.V., CONNOLLY, D.T. & DELARCO, J.E. (1991). Specific binding of vascular permeability factor to endothelial cells. *Biochem. Biophys. Res. Commun.*, **175**, 68–76.

RAMPART, M. & WILLIAMS, T.J. (1986). Polymorphonuclear leukocyte-dependent plasma leakage in the rabbit skin is enhanced or inhibited by prostacyclin, depending on the route of administration. *Am. J. Pathol.*, **124**, 66–73.

SENGER, D.R., GALLI, S.J., DVORAK, A.M., PERRUZZI, C.A., HARVEY, V.S. & DVORAK, H.F. (1983). Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. *Science*, **219**, 983–985.

SENGER, D.R., PERRUZZI, C.A., FEDER, J. & DVORAK, H.F. (1986). A highly conserved vascular permeability factor secreted by a variety of human and rodent tumor cell lines. *Cancer Res.*, **46**, 5629–5632.

SENGER, D.R., CONNOLLY, D.T., VAN DE WATER, L., FEDER, J. & DVORAK, H.F. (1990). Purification and NH_2-terminal amino acid sequence of guinea pig tumor-secreted vascular permeability factor. *Cancer Res.*, **50**, 1774–1778.

VAISMAN, N., GOSPODAROWICZ, D. & NEUFELD, G. (1990). Characterization of the receptors for vascular endothelial growth factor. *J. Biol. Chem.*, **265**, 19461–19466.

WEDMORE, C.V. & WILLIAMS, T.J. (1981). Control of vascular permeability by polymorphonuclear leukocytes in inflammation. *Nature*, **289**, 646–650.

WILLIAMS, T.J. (1982). Vasoactive intestinal polypeptide is more potent than prostaglandin E_2 as a vasodilator and oedema potentiator in rabbit skin. *Br. J. Pharmacol.*, **77**, 505–509.

WILLIAMS, T.J. & MORLEY, J. (1973). Prostaglandins as potentiators of increased vascular permeability in inflammation. *Nature*, **246**, 215–217.

WILLIAMS, T.J. & PECK, M.J. (1977). Role of prostaglandin-mediated vasodilatation in inflammation. *Nature*, **270**, 530–532.

WILLIAMS, T.J. & PIPER, P.J. (1980). The action of chemically pure SRS-A on the microcirculation *in vivo*. *Prostaglandins*, **19**, 779–789.

(Received November 4, 1992
Revised December 23, 1992
Accepted January 19, 1993)