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Vascular Permeability Factor: A Unique Regulator of Blood Vessel Function

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Abstract Vascular permeability factor (VPF), also known as vascular endothelial growth factor (VEGF), is a potent polypeptide regulator of blood vessel function. VPF promotes an array of responses in endothelium, including hyperpermeability, endothelial cell growth, angiogenesis, and enhanced glucose transport. VPF regulates the expression of tissue factor and the glucose transporter. All of the endothelial cell responses to VPF are evidently mediated by high affinity cell surface receptors. Thus, endothelial cells have a unique and specific spectrum of responses to VPF. Since each of the responses of endothelial cells to VPF are also elicited by agonists, such as bFGF, TNF, histamine and others, it remains a major challenge to determine how post-receptor signalling pathways maintain both specificity and redundancy in cellular responses to various agonists.

Key words: vascular endothelial growth factor, endothelium, angiogenesis

The biochemical literature provides numerous examples of bioactive substances that have been purified independently by different laboratories using different biochemical assays. In many instances, the substances were named according to a particular function. A recent case in point is the discovery of the potent vasoactive protein known as *vascular permeability factor* (VPF) (Senger et al., 1983, 1990; Connolly et al., 1989a,b), *vascular endothelial growth factor* (VEGF) (Ferrara and Henzel, 1989; Gospodarowicz et al., 1989; Conn et al., 1990), and *vasculotropin* (Plouet et al., 1989). VPF has been independently purified from cells in culture and characterized by at least six different groups using three different and unrelated bioassays. VPF was first purified based upon its permeability-enhancing effects on blood vessels (Senger et al., 1983, 1990; Connolly et al., 1989a,b), but was later independently purified using mitogenic assays (Ferrara and Henzel, 1989; Gospodarowicz et al., 1989; Conn et al., 1990b; Plouet et al., 1989) and again using a coagulation assay (Clauss et al., 1990). Which, if any, of the activities of VPF are the most physiologically relevant? The answer to this question is not clear at this time, but it seems likely that, like many other cytokines, VPF can have diverse effects that depend on the specific biological context in which it is found.

VPF was described by Dvorak and coworkers as a widely distributed tumor-derived protein (Senger et al., 1983, 1986). It was proposed that the permeability-enhancing activity of VPF could contribute to the abnormal properties of tumor-associated blood vessels. Hyperpermeability of the tumor vasculature (Gerlowski and Jain, 1986) would promote the leakage of plasma proteins into the tumor tissue, the accumulation of fibrin, the formation of characteristic tumor stroma, and neovascularization (Dvorak et al., 1981). The expression of VPF in the tumor environment could thus contribute to the wound-like properties of tumors (Dvorak, 1986; Whalen, 1990).

Is the permeability-enhancing activity of VPF biologically relevant? Two lines of evidence suggest that the answer to this question is yes. First, an antibody against VPF was found to block the accumulation of ascitic fluid by tumor-bearing guinea pigs (Senger et al., 1983). Second, the permeability-enhancing activity of VPF is greater or equipotent in comparison with other well-characterized mediators of vascular permeability (Table I). In fact, VPF's potency is approximately 1,000 times that of histamine when tested in guinea pig skin (Senger et al., 1983). It

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TABLE I. Comparison of VPF With Other Permeability Mediators

Mediator	Effective concentration (M)
ADP, adenosine, inosine[a]	10^{-5}
Histamine, serotonin[a]	10^{-6}
Bradykinin, substance P[a]	10^{-7}
Prostaglandins E1, E2, F2α[a]	$> 10^{-8}$
Leukotrienes C4, D4, E4, B4[a]	$> 10^{-9}$
C3a, C5a[a]	$> 10^{-9}$
Platelet activating factor[a]	$> 10^{-9}$
Plasma kallikrein[b]	10^{-9}
VPF[c]	10^{-9}

[a]Svensjo and Grega, 1986.
[b]Imamura et al., 1984.
[c]Senger et al., 1983; Connolly et al., 1989a,b.

is evident from these data that the regulation of vascular permeability can be influenced by numerous mediators whose specific functions depend upon the specific biological context in which they are found. It therefore is appropriate to include VPF on the list of regulators of vascular permeability.

After VPF was purified, we observed that it is a potent endothelial cell mitogen (Connolly et al., 1989a,b). At about the same time, several other groups independently used endothelial cell mitogenic assays to purify VEGF (Ferrara and Henzel, 1989; Gospodarowicz et al., 1989; Conn et al., 1990b) and vasculotropin (Plouet et al., 1989). Comparisons of the physical properties of the proteins, amino acid sequences, and sequences obtained from cDNA clones indicate that VEGF and vasculotropin are evidently identical to VPF (Keck et al., 1989; Leung et al., 1989; Conn et al., 1990a; Plouet et al., 1989), and share distant structural homology to the platelet-derived growth factor/v-sis family of proteins. Different monomeric isoforms containing 189, 165, and 121 amino acids can arise from differential mRNA splicing (Leung et al., 1989; Tischer et al., 1989). Since VPF is a disulfide-linked dimer, it is likely that heterodimer formation could lead to some of the structural heterogeneity observed in natural VPF (Connolly et al., 1989a,b; Senger et al., 1990). The functional significance of this heterogeneity is not presently understood, nor is it known if the different homodimeric or heterodimeric isoforms of VPF have different biological properties.

Is VPF-induced endothelial cell mitosis biologically relevant? To answer this query, it is useful to compare the potency of VPF with other known endothelial cell mitogens. Table II shows that the EC_{50} for VPF-stimulated endothelial cell growth in vitro compares favorably with other growth factors. Furthermore, VPF has been shown to promote angiogenesis in rat cornea and chicken chorioallantoic membrane models (Connolly et al., 1989a; Leung et al., 1989; Plouet et al., 1989). These data suggest that VPF could directly contribute to the regulation of angiogenesis by promoting endothelial cell growth. This might be expected to occur during normal or malignant development, during wound healing, or during other as yet unidentified pathological conditions.

Are the effects of VPF limited to the endothelium? The mitogenic effect of VPF has thus far been found to be highly specific for endothelial cells in vitro (Connolly et al., 1989a; Ferrara and Henzel, 1989; Gospodarowicz et al., 1989; Conn et al., 1990b; Plouet et al., 1989). However, it should be emphasized that the overall spectrum of bioactivity for VPF is probably not limited to vascular endothelium. Even if the endothelium is the major target of VPF, enhanced vascular

TABLE II. Comparison of VPF With Other Endothelial Cell Growth Factors

Growth factor	IC_{50} (pM)	Endothelial cells	Reference
EGF	800	bovine pulmonary	Schreiber et al., 1986
TGFα	600	bovine pulmonary	Schreiber et al., 1986
PD-ECGF	440	porcine aortic	Miyazono et al., 1987
aFGF[a]	190	bovine aortic	Schreiber et al., 1985
bFGF[b]	3.1	bovine aortic	Gospodarowicz et al., 1984
VPF[c]	2.5	bovine aortic	Connolly et al., 1989
	1.6	adrenal capillary	Gospodarowicz et al., 1989

[a]aFGF is also referred to as endothelial cell growth factor and heparin-binding growth factor 1. The IC_{50} reported was in the presence of heparin which enhanced activity.
[b]bFGF is also referred to as heparin-binding growth factor 2.
[c]VPF is also referred to as VEGF and vasculotropin.

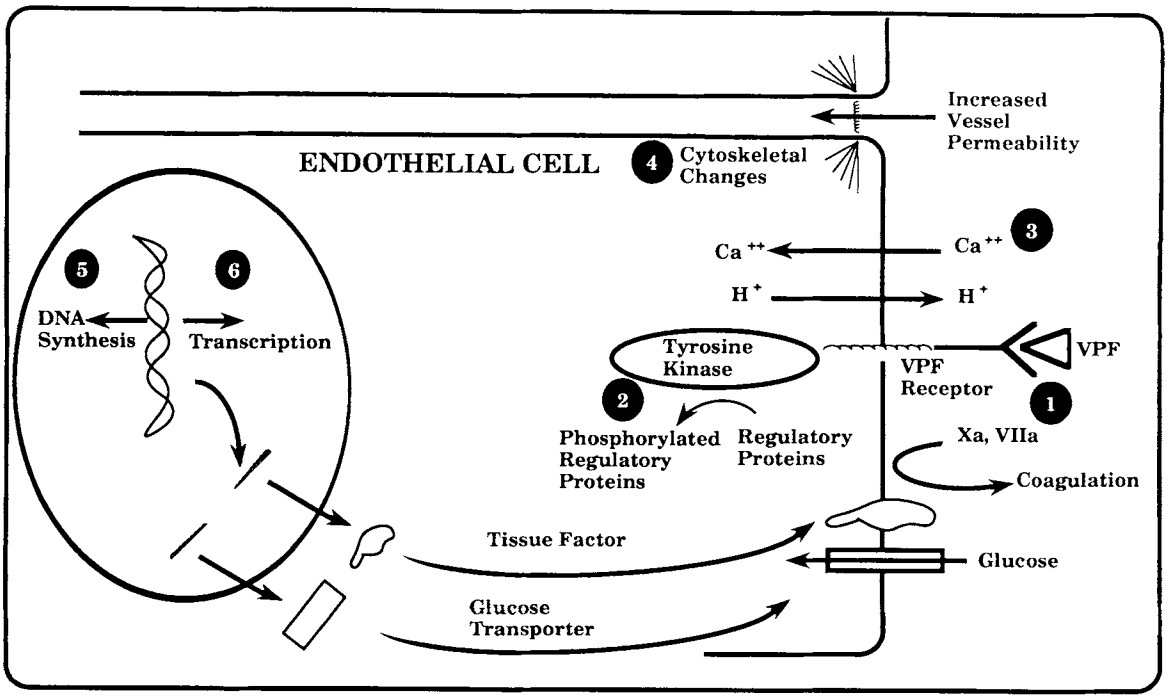

Fig. 1. Model for the interaction of VPF with endothelial cells. 1: VPF binds to high-affinity cell surface receptors. 2: Tyrosine kinase activity, presumably associated with the receptor itself, is activated upon VPF binding. Regulatory proteins are phosphorylated on tyrosine residues. 3: Intracellular Ca^{2+}, pH, and inositol trisphosphate increase. 4: Cytoskeletal changes occur that lead to increased vascular permeability. 5: DNA synthesis and mitosisis are initiated. 6: The genes for tissue factor and glucose transporter are activated. Glucose transport is increased. Tissue factor can interact with coagulation factors to initiate coagulation.

permeability could functionally compromise such specificity indirectly in vivo. For example, leakage of plasma proteins could lead to extravascular coagulation that in turn could initiate other related extravascular events. Extravasation of plasma growth factors or chemoattractants could attract leukocytes or monocytes. Finally, it has been shown that VPF itself is a potent chemoattractant for monocytes (Clauss et al., 1990). As with other cytokines, it is difficult to predict all of the in vivo effects of VPF in specific situations. Parameters such as local concentrations, the specific cell types involved, and interactions with other cytokines are likely to modulate the effects of VPF.

Some of the activities of VPF can be considered to be proinflammatory. For example, enhanced vascular permeability is one of the earliest events in the inflammatory response (Ryan and Majno, 1977). Also, VPF is a chemoattractant for monocytes (Clauss et al., 1990). Finally, VPF has been shown to enhance the activity of the inflammatory mediator tumor necrosis factor (TNF) in the regulation of the expression of at least two endothelial cell proteins. Tissue factor (thromboplastin), a key component in the initiation of coagulation (Nemerson, 1988), is not normally expressed by endothelial cells, but can be induced on endothelium by inflammatory mediators (Cotran, 1987). VPF or TNF independently stimulate tissue factor expression by endothelial cells, but the two cytokines together are more effective in initiating the pro-coagulant response (Clauss et al., 1990). Likewise, glucose transport and GLUT-1 glucose transporter gene expression are enhanced by either VPF or TNF alone, but more effectively by the addition of both cytokines together (Pekala et al., 1990). This could be important at sites of inflammation, which are often hypoxic and where enhanced glycolysis could be important. In contrast, TNF completely blocks the mitogenic response of endothelial cells to VPF (Pekala et al., 1990). This latter result emphasizes that even though the activities of these cytokines overlap, the overall spectrum of activities of VPF and TNF is distinct.

How does VPF elicit the wide array of cellular responses outlined above? A model for VPF interaction with endothelial cells is shown in Figure 1. VPF interacts with high affinity receptors on endothelial cells (Vaisman et al., 1990; Plouet

and Moukadiri, 1990; Olander et al., 1991). We have recently obtained preliminary evidence that tyrosine-specific protein kinase activity is associated with VPF receptors (J. Huang and D. Connolly, unpublished observations). The earliest *signal transduction* event following VPF binding is presumably phosphorylation of tyrosine residues, but substrates for the kinase have not been identified. Secondary events include increases in inositol trisphosphate, intracellular calcium (Brock et al., 1991; Criscuolo, et al., 1989), and intracellular pH (R. Worthington, unpublished observations). Cytoskeletal changes then presumably lead to cellular contraction and increased vascular permeability. von Willebrand factor is released from Weibel-Palade bodies (Brock et al., 1991). Changes in gene expression occur in response to VPF, notably in the tissue factor (Clauss et al., 1990) and GLUT-1 glucose transporter genes (Pekala et al., 1990). DNA synthesis and mitosis occur. Obviously, there are large gaps in our knowledge regarding the specific details of all of these steps.

Many of the effects of VPF on endothelial cells are shared by other agonists. For example, bFGF stimulates endothelial cell growth. However, bFGF is not a permeability mediator. Histamine is a potent permeability mediator, but is not an endothelial cell mitogen. TNF, like VPF, stimulates glucose transporter and tissue factor expression; but unlike VPF, TNF inhibits endothelial cell growth. One of the major challenges for future biochemical research in this area will be to unravel the complicated post-receptor pathways that lend specificity, but at the same time lend redundancy, to the interactions of these cytokines with their target cells.

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A human gene for VEGF (VPF) containing eight exons has been described by Tischer, E., Mitchell, R., Hartman, T., Silva, M., Gospodarowicz, D., Fiddes, J.C., and Abraham, J.A. (1991). J. Biol. Chem. 266:11947–11954. VEGF (VPF) was shown to stimulate tyrosine phosphorylation of a 190 kDa polypeptide in endothelial cells [Myoken, Y., Kayada, Y., Okamoto, T., Kan, M., Sato, G.H., and Sato, J.D. (1991). Proc. Natl. Acad. Sci. USA 88:5819–5823].

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