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COMMENTARY

VASOACTIVE EFFECTS OF GROWTH FACTORS

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Vascular tone in the intact organism is regulated by a variety of extracellular factors including circulating cations, vasoactive hormones and blood pH [1, 2]. Local neuronal influence and local production of vasoactive agonists also may play important roles in vascular smooth muscle cell (VSMC†) function [3]. Although the primary function of VSMC is contraction and maintenance of tone, in certain conditions, such as atherosclerosis, VSMC proliferate and migrate. In this disease, VSMC appear to have abnormal vasoreactivity [4]. This concept is based on findings of increased resting tone [5], increased frequency of coronary artery spasm at atherosclerotic lesions [6], and hypercontractile responses to agonists such as ergonovine, histamine and serotonin [7, 8] and paradoxical constriction to acetylcholine [9]. Based on this association between proliferative state and abnormal VSMC function, we proposed that mitogenic influences present in the atherosclerotic vessel may contribute to the enhanced contractile activity observed at sites of atherosclerosis [10, 11]. This proposal was predicted in part on the data that vasoactive agonists and growth factors share membrane signalling mechanisms involving phosphoinositide hydrolysis and calcium mobilization [12] that are associated with the induction of contraction in vascular smooth muscle [13]. The formation of atherosclerotic lesions likely involves the action of a number of growth factors. We focused our studies on two well characterized VSMC mitogens: platelet-derived growth factor (PDGF) and epidermal growth factor (EGF). Therefore, we [10, 11] and others [14–18] have tested the contractile effects of PDGF and EGF in several *in vivo* and *in vitro* vascular models.

Phasic contraction

The effect of human PDGF on contractile tension in isolated rat aortic strips is illustrated in Fig. 1A. PDGF produced a concentration-dependent contractile response with an EC_{50} of approximately 30 pM. The contraction was readily reversible after incubation for periods as long as 30 min. It did not exhibit marked tachyphylaxis nor was it affected by ketanserine, phentolamine or indomethacin [11]. Denudation of endothelium did not alter the response, suggesting that the effect was specific for PDGF receptors present on VSMC.

EGF-induced contractions in rat aorta or ileocolic artery are similar to those found with PDGF [10, 16]. The EC_{50} for contraction is 19 nM in aorta and 1.7 nM in ileocolic artery. As with PDGF, the development of tension in response to EGF is slow, requiring 3–5 min to reach peak contractile tone. Unlike PDGF, the contractile response to EGF in ileocolic artery is abolished by indomethacin [16]. These data indicate that a cyclooxygenase pathway product (probably $PGF_{2\alpha}$) may mediate some of the EGF response. In summary, growth factor-stimulated rat vessel contraction is potent, independent of endothelium, and associated with a slow onset of contraction. Although vasoconstriction appears to be a common effect of growth factors (basic fibroblast growth factor also stimulated rat aortic contraction, see Ref. 11), it appears that PDGF and EGF may stimulate contraction, at least in some vessels, via different mechanisms.

To understand potential mechanisms involved in growth factor-mediated vasoconstriction, a brief review of the contractile machinery is necessary [20]. The common mechanism underlying vasoconstrictor action is a transient rise in $[Ca^{2+}]_i$, which regulates actin–myosin interactions. Briefly, the increase in $[Ca^{2+}]_i$ results in formation of Ca^{2+}–calmodulin complexes that bind to and activate the myosin light chain kinase (MLCK). The MLCK then phosphorylates myosin light chains that catalyze actomyosin ATPase activity resulting in cross bridge formation and contraction. When $[Ca^{2+}]_i$ decreases below 100 nM, the Ca^{2+}–calmodulin–MLCK complex dissociates from the myosin light chain and phosphatase activity causes dephosphorylation of myosin and relaxation. However, in tonic smooth muscle contraction, tension is maintained despite relatively low $[Ca^{2+}]_i$ and lack of myosin phosphorylation. The sustained maintenance of tone with low energy expenditure has been explained by the "latch bridge" hypothesis [21].

Numerous hormones, neurotransmitters and growth factors have been shown to generate a common series of intracellular biochemical signals upon binding to their receptors. Angiotensin II, alpha-adrenergic agonists, and other vasoactive hormones (e.g. alpha-thrombin, serotonin, bradykinin, and vasopressin) stimulate an increase in $[Ca^{2+}]_i$ as part of the initial phase of contraction [22–26]. Recent

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† Abbreviations: $[Ca^{2+}]_i$, intracellular free Ca^{2+} concentration; EGF, epidermal growth factor; IP_3, inositol trisphosphate; MLCK, myosin light chain icinase; PDGF, platelet-derived growth factor; pH_i, intracellular pH; PKC, protein kinase C; and VSMC, vascular smooth muscle cell(s).

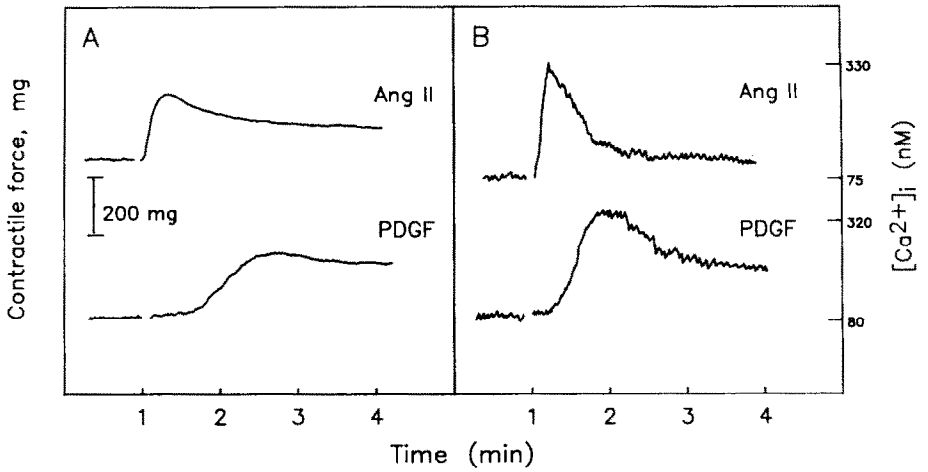

Fig. 1. PDGF and angiotensin II-stimulated VSMC responses. (A) Contraction of isolated rat aorta induced by PDGF or angiotensin II (Ang II). Helical strips of rat aorta with intact endothelium were prepared as previously described [10, 11]. Tracings represent typical contractile responses to addition (time = 1 min) of 10 nM Ang II or 3 ng/ml (approximately 90 pM) PDGF. (B) Changes in $[Ca^{2+}]_i$ in suspensions of rat aortic VSMC induced by PDGF or Ang II. Fura-2 loading, fluorescence measurements, and determination of $[Ca^{2+}]_i$ were performed as previously described [19]. Tracings represent typical responses of fura-2 fluorescence to addition (time = 1 min) of 3 nM Ang II or 1.0 ng/ml purified PDGF.

work has demonstrated that this response is mediated by activation of phospholipase C that hydrolyzes phosphotidylinositol bisphosphate (PIP_2) generating diacylglycerol (DG) and inositol trisphosphate (IP_3), the latter releasing intracellular calcium stores [27, 28]. For vasoconstrictors such as angiotensin II, the majority of the initial rise in $[Ca^{2+}]_i$ is mediated by release of intracellular calcium stores [29]. However, angiotensin II stimulates an increase in Ca^{2+} influx [25, 30] and in the absence of extracellular Ca^{2+} the elevated plateau phase of $[Ca^{2+}]_i$ which persists for several minutes following agonist binding is abolished [29]. Other vasoconstrictors are more dependent on extracellular Ca^{2+}; the increase in $[Ca^{2+}]_i$ following $alpha_{1a}$ receptor stimulation, for example, is totally dependent on extracellular Ca^{2+} [31, 32]. The precise roles of VSMC voltage-dependent, dihydropyridine-sensitive calcium channels and ligand-operated calcium channels in mediating agonist-stimulated Ca^{2+} influx are unclear at this time [31, 33, 34]. However, as detailed below, for certain vasoconstrictors such as the phorbol esters [35, 36], Ca^{2+} influx via dihydropyridine-sensitive channels appears to be critical to the contractile response.

PDGF is similar to angiotensin II in that it has been shown to increase IP_3 formation and $[Ca^{2+}]_i$ in several systems [37, 38]. However, the time course for PDGF-induced Ca^{2+} mobilization is considerably slower than for other agonists such as angiotensin II in VSMC ([11, 39]; see Fig. 1B) or vasopressin in fibroblasts [40]. The magnitude of IP_3 formation is also significantly smaller than observed with these other agonists ([40]; B. Berk, unpublished observations). In cultured VSMC, the rise in $[Ca^{2+}]_i$ induced by PDGF is inhibited by 75–80% in the absence of extracellular Ca^{2+}, whereas the angiotensin II response is inhibited only by about 40% [11]. These data suggest that the rise in $[Ca^{2+}]_i$ induced by PDGF may not be related predominantly to IP_3-mediated release of intracellular calcium stores. Pathways which may be involved include phosphorylation events mediated either by the PDGF receptor tyrosine kinase or by the calcium and phospholipid-dependent protein kinase C (PKC) stimulated by PDGF [38]. The rise in intracellular pH (pH_i) following PDGF stimulation of Na^+/H^+ exchange [41] may alter $[Ca^{2+}]_i$ perhaps by release of intracellular calcium stores [42] or by enhancement of IP_3-mediated calcium release. Surprisingly, when EGF is administered to cultured VSMC, there is no rise in $[Ca^{2+}]_i$ even though EGF stimulates a small increase in the rate of Ca^{2+} efflux [10], activates Na^+/H^+ exchange (B. Berk, unpublished observations), and stimulates VSMC growth [10].

These differences in Ca^{2+} mobilization may play a role in explaining the differences in the initial rate of contraction induced by classic vasoconstrictor agonists and growth factors. It is apparent upon comparison of the PDGF and angiotensin II contractile responses shown in Fig. 1 that there is a significant time lag for the PDGF-induced contraction. While maximal force development in response to PDGF occurred within 5 min and was equivalent to 40% of that observed in response to angiotensin II, the time course of tension development showed that for a given amount of tension the rate of contraction was always slower for PDGF than for angiotensin II. For example, the time required to reach 150 mg of force was about 120 sec for PDGF as opposed to 13 sec for angiotensin II. A similar time delay has been observed for EGF contractions [17]. These findings suggest that, although intracellular release of Ca^{2+} may be an important mechanism for growth factor-stimulated contraction, it is unlikely to be the only mechanism present. Other mechanisms potentially involved include effects of growth factors on Na^+ flux, intra-

cellular pH, and phosphorylation mediated either by tyrosine kinases or protein kinase C.

Tonic contraction

Several lines of evidence suggest that sustained agonist-induced contractions depend on Ca^{2+} influx and likely involve activation of PKC by diacylglycerol (DG) and Ca^{2+}. Phorbol esters, which directly activate PKC, stimulate rabbit and rat aortic strip contractions [35, 36]. These contractions are dependent on extracellular Ca^{2+} and are inhibited by dihydropyridines, suggesting that a significant amount of the Ca^{2+} influx is mediated by voltage-dependent Ca^{2+} channels. Furthermore, addition of the Ca^{2+} channel agonist BAY K 8644 or depolarization with potassium augments PKC-stimulated contraction [32, 43]. Several investigators have demonstrated altered Ca^{2+} channel activity following stimulation with protein kinase C, with both increases and decreases in the slow, L-type voltage-activated conductance [44, 45]. This suggests that PKC-mediated phosphorylation of the L-type channel or of a regulatory protein may be one mechanism for PKC-mediated contraction.

Because EGF and PDGF have been shown to stimulate PKC in many cell systems, it is likely that activation of PKC is important in growth factor-mediated vasoactive effects in VSMC. Our laboratory has generated preliminary data that PDGF and EGF stimulate the phosphorylation of an 80,000 mol. wt acidic protein in VSMC* which is reported to be a PKC substrate in a variety of cell systems [46]. Thus, one possible mechanism for growth factor-mediated vasoconstriction may involve activation of PKC followed by stimulation of Ca^{2+} influx. This appears to be important particularly in the maintenance of tonic contraction.

Protein kinase C may regulate tonic contraction through several pathways. By phosphorylating the voltage-dependent Ca^{2+} channel, protein kinase C may increase Ca^{2+} channel conductance resulting in enhanced Ca^{2+} entry as discussed above. *In vitro*, protein kinase C has been shown to phosphorylate the myosin light chain either isolated or incorporated into heavy meromyosin or myosin [47]. Although phosphorylation by PKC alone does not appear to affect the enzymatic properties of myosin, in combination with MLCK there is a reduction in actin-activated ATPase activity due to an increase in the K_m for actin [48]. It is unclear at this time what the *in vivo* correlate of these phosphorylations would be. Rasmussen *et al.* [49] have suggested that PKC-mediated phosphorylation of the filamin–actin–desmin system may alter intermediate filament interactions such as bundling which may regulate tonic smooth muscle functions. PKC also activates a plasma membrane Na^+/H^+ exchange mechanism in many cells [38], including VSMC [19, 50]. Activation of this exchange mechanism causes intracellular alkalinization and a rise in intracellular Na^+, ionic events that may alter vasoreactivity significantly.

Intracellular pH has important effects on vessel contractile tone. In general, alkalinization causes vasoconstriction, whereas acidification causes vasodilation [1, 2, 51]. A role for Na^+/H^+ exchange has been postulated based on findings that the Na^+/H^+ exchange inhibitor, amiloride, blocks phorbol ester-induced contraction of rat aorta [36]. Administration of NH_4Cl, which causes an increase in pH_i, is associated with slow development of a contractile response in rat aorta under basal conditions.† In the presence of pre-existing tone, however, NH_4Cl causes an immediate transient relaxation [52]. Thus, a rise in pH_i due to activation of Na^+/H^+ exchange may be involved in contraction, although the relationship is complex.

The possible mechanisms by which alterations in pH_i may influence VSMC force development have been reviewed recently by Wray [53]. For example, purified preparations of actin–myosin develop less force in acidic solutions consistent with decreased ATPase activity [54], suggesting that the converse would be true in a more alkaline environment. Myofibrillar Ca^{2+} sensitivity is pH dependent with a decrease in the K_d of calmodulin for Ca^{2+} by an order of magnitude as pH increases from 6.5 to 7.5 [55]. Competition between H^+ and Ca^{2+} for intracellular binding sites may lead to opposing effects on contractile tone. Displacement of Ca^{2+} bound to myofilaments by H^+ would decrease tone, while increased pH would promote binding of Ca^{2+} to myofilaments and enhance tension [56]. Finally, alterations in receptor sensitivity and processing, as well as in the duration and magnitude of second messenger signalling, may be regulated by pH_i. Receptor processing is pH dependent and, for certain ligands, intracellular acidification has been shown to be associated with termination of the response [57]. Thus, a sustained rise in pH_i may contribute to tonic vasoconstrictor action by preventing tachyphylaxis due to receptor downregulation. The activity of purified phospholipase C has been shown to have a strong pH dependency [58]. Griendling *et al.* [59] found that alkalinizing VSMC (with 5 mM NH_4Cl) increases angiotensin II stimulated DG formation by 80% at 5 min, while acidifying the cells (with 20 mM potassium acetate) inhibits DG formation. These data suggest that the increase in pH_i following agonist stimulation may be important in sustained generation of DG from phosphatidylinositol. Because PKC is dependent on DG for activation by Ca^{2+}, this pH-dependent generation of DG may allow continued PKC activity at only minimally elevated $[Ca^{2+}]_i$. This formulation would explain several reports indicating only minimal elevation of $[Ca^{2+}]_i$ in association with phorbol ester induced contractions [60].

Stimulation of the Na^+/H^+ exchanger also causes a significant increase in Na^+ influx. As postulated by Blaustein [61], the presence of Na^+/Ca^{2+} exchange in VSMC could effect an increase in $[Ca^{2+}]_i$ when the transmembrane Na^+ gradient is decreased such as would occur with an increase in intracellular Na^+. We [62] and others [63] have shown recently that cultured rat aortic smooth muscle cells possess a Na^+/Ca^{2+} exchange mechanism. Following binding of agonists such as angiotensin II or PDGF which activate Na^+/H^+ exchange and release of Ca^{2+}

* T. Tsuda, unpublished observations, cited with permission.

† N. R. Danthuluri, personal communication, cited with permission.

stores, there are increases in both intracellular Ca^{2+} and Na^+. Stimulation of Na^+ influx via the Na^+/H^+ exchanger would tend to decrease Ca^{2+} efflux via Na^+/Ca^{2+} exchange and may result in prolongation of the rise in $[Ca^{2+}]_i$. Although the physiologic importance of this mechanism is unclear, several investigators have found contractions induced in low Na^+ solutions [51, 64]. Therefore, growth factor-stimulated Na^+/H^+ exchange, by virtue of stimulating increases in intracellular pH and intracellular Na^+, may contribute to tonic contraction.

Growth factor-mediated vasodilation

Although EGF and PDGF consistently stimulate contraction of rat arteries, several *in vivo* and *in vitro* studies by Hollenberg and collaborators [17, 18] in canine vessels have demonstrated EGF-mediated vasodilation or inhibition of vasoconstrictor responses. In isolated canine helical mesenteric strips, EGF alone did not affect resting tone, but contractile responses to norepinephrine, KCl or transmural electrical stimulation were inhibited up to 50% by EGF. The EGF-mediated effect occurred with an EC_{50} of approximately 1 nM, was maximal after 15 min of pretreatment, did not require intact endothelium and was not inhibited by indomethacin [17]. Close-arterial administration of EGF into canine femoral, superior mesenteric, coronary, celiac and renal vascular beds caused a significant increase in blood flow [18]. The response to EGF was maximal within 2–6 min and significantly delayed in comparison to vasodilators such as nitroprusside or isoproterenol which cause maximal dilation within 1 min. This delay is reminiscent of the time delay for EGF-mediated rat aorta contraction described above. There were significant differences in vascular bed responsiveness to EGF with the femoral and superior mesenteric vessels demonstrating increases in flow up to 70%, while the other vessels had only a 20% flow increase. Blockade of sympathetic, cholinergic or histaminergic receptors did not affect the response. Of interest is the finding that EGF-mediated canine vessel dilation was not inhibited by indomethacin, whereas rat vessel contraction was abolished.

Although PDGF has been studied less extensively than EGF, administration to canine coronary arteries [15] and rat cerebral vessels [14] has been associated with decreased contractile tension. These findings indicate a varied profile for PDGF- and EGF-mediated vascular effects, with responses differing among vascular beds and species.

Other vasoactive growth factors

Although this discussion has focused on the potent VSMC mitogens EGF and PDGF, it is clear that there are several other agonists that have mitogenic and vasoactive properties. Serotonin, like PDGF, is released by aggregating platelets and is important in mediating contractions in the absence of endothelium [65]. Under certain conditions, serotonin can induce an endothelium-dependent relaxation of isolated coronary arteries which appears to be mediated by prostaglandin production [66, 67]. At concentrations known to stimulate contraction, serotonin also stimulates VSMC cell division [68] although the precise mechanism is unclear.

Interleukin 1 is a polypeptide primarily secreted by macrophages, but recently demonstrated to be produced by both endothelial cells and VSMC [69]. Interleukin 1 has a multitude of effects on the vascular wall including increases in leukocyte adherence to endothelium [70] and synthesis of PGE_2, PGI_2, and platelet-activating factor [71], which are potent vasodilators. Thus, when administered intravenously, interleukin 1 induces a prompt but reversible fall in arterial pressure [72]. Interleukin 1 is a growth factor for VSMC [73], although this effect is revealed only in the presence of indomethacin because of production of growth inhibitory prostaglandins. It is of interest that the structure of acidic and basic fibroblast (endothelial cell) growth factors are related to interleukin 1, suggesting a role for interleukin 1 in modulating both endothelial and VSMC growth responses that may be involved in atherosclerosis [69].

Summary and implications

Growth factor-mediated vasoconstriction ultimately must involve alterations in $[Ca^{2+}]_i$. For PDGF a phospholipase C, IP_3-dependent mechanism has been demonstrated, while the nature of EGF-mediated effects on VSMC $[Ca^{2+}]_i$ is unclear. The difference in time course for Ca^{2+} mobilization and contraction by the growth factors compared to more classic agonists such as angiotensin II suggests that other pathways may predominate. Activation of PKC is common to PDGF and EGF, suggesting that PKC-mediated effects such as increased Ca^+ channel conductance [38] or phosphorylation of cytoskeletal proteins [48, 74] may represent one pathway which is independent of phospholipase C. Both the PDGF and EGF receptors have tyrosine kinase activity which has been implicated in the PKC-independent actions [75] of these mitogens. Growth factor-stimulated Na^+/H^+ exchange has been shown to involve both PKC-dependent and independent mechanisms. Alkalinization via its effects on the contractile machinery may increase sensitivity to Ca^{2+} [53], while a rise in Na^+ may increase $[Ca^{2+}]_i$ via effects on Na^+/Ca^{2+} exchange [62, 63]. Finally, PDGF and EGF increase arachidonic acid metabolism and various cyclo-oxygenase products which appear to mediate vasoconstriction in response to EGF [16], but not PDGF [11].

All of the growth factors discussed also cause vasodilation. A likely mechanism for growth factor-mediated vasodilation is enhanced arachidonic acid metabolism with generation of vasodilatory prostaglandins by either endothelial cells or VSMC. This mechanism appears to be responsible for interleukin 1 mediated hypotension and serotonin-induced vasodilation, both of which can be inhibited by pretreatment with indomethacin [65, 72]. However, EGF-mediated vasodilation in the dog is not affected by indomethacin [18]. Thus, at this time the mechanisms underlying EGF- and PDGF-mediated vessel relaxation are unknown.

Several physiological roles may be considered for growth factor-stimulated vasoactivity. At sites of active platelet degranulation such as the thrombus associated with acute coronary artery syndromes,

EGF, PDGF, and serotonin released from platelets may cause acute changes in tone. In keeping with the emphasis placed above on the tonic nature of growth factor-mediated vasoactive effects, it is attractive to postulate that during accelerated atherosclerosis, as manifest by VSMC proliferation, growth factors may contribute to the abnormal vasoreactivity of atherosclerotic vessels. This hypothesis is supported by recent findings that atherosclerotic VSMC express increased levels of PDGF A chain mRNA [76], indicating that autocrine/paracrine effects of PDGF may modulate VSMC function under these circumstances.

Local generation of vascular mediators has been demonstrated for the renin–angiotensin system [3] and may be involved in regulation of vascular tone in several vascular beds. Local production of PDGF may be important in the enhanced vasoreactivity of atherosclerosis especially if there is differential expression of the various dimers (AA, AB, and BB) which themselves may have differing effects on tone, VSMC growth and vasoreactivity. Hollenberg and colleagues have suggested that production of EGF or the closely related α-transforming growth factors may mediate local vasodilation [18]. Because α-transforming growth factors are synthesized by certain tumors [77], it is possible that local blood flow in some malignancies may be regulated in a paracrine manner.

The preliminary findings summarized here for the effects of EGF, PDGF, serotonin and interleukin 1 in a variety of vascular beds indicate a wide range of arterial responsiveness to vascular smooth muscle cell mitogens. Coupled with the potential role of local production of vasoactive mediators, one may propose that growth factors will have important roles in modulating vessel tone and vasoreactivity in both normal and diseased states. The magnitude of their role in human disease is unclear at this time, but this should be an area of fruitful investigation.

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