Regulated Expression of the Platelet-derived Growth Factor A Chain Gene in Microvascular Endothelial Cells*

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Platelet-derived growth factor (PDGF) is composed of homologous polypeptide chains, termed A and B, that are expressed as mitogenically active A-A, B-B, or A-B dimers. Previous work in our laboratory has demonstrated that PDGF B chain mRNA expression is stimulated in microvascular endothelial cells by phorbol esters (PMA), thrombin, and transforming growth factor-β (TGF-β) and blocked by agents that elevate cyclic AMP (cAMP). Here we report the first evidence that the expression of A chain mRNA is also regulated in these cells. PDGF A chain mRNA levels were increased 5–25-fold by phorbol esters, thrombin, and TGF-β. Transcripts of four different sizes were induced. The increase in A chain mRNA stimulated by TGF-β was more prolonged (peak 4 h, duration 48 h) than the increase stimulated by PMA and thrombin (peak 4 h, duration 8 h). Among the agents known to increase B chain mRNA levels, PMA was most efficacious, followed in decreasing order by thrombin and TGF-β. However, for A chain mRNA induction by these same agents, the order was reversed; TGF-β was most efficacious, followed in decreasing order by thrombin and PMA. Agents that elevate cyclic AMP, known to block induction of B chain mRNA, blocked A chain induction by thrombin but had less effect on A chain mRNA induced by TGF-β. Thus PDGF A chain mRNA levels are regulated by the same agents that regulate B chain mRNA levels in microvascular endothelial cells. While the changes in A chain mRNA are qualitatively similar to the changes in B chain mRNA in microvascular endothelial cells, there are differences in the relative efficacies of these agents in the regulation of PDGF A and B chain genes. These differences suggest that the forms of PDGF produced by endothelial cells depend on the nature of the inducing stimulus.

PDGF purified from human platelets is a 32-kDa heterodimer composed of two homologous disulfide-linked polypeptide chains, termed A and B, that are encoded by separate genes (1–3). Several cell types other than human platelets express A-A or B-B homodimers, and it is possible that some tissues express multiple forms of PDGF (4–10). PDGF produced by endothelial cells is thought to be important in mediating vascular responses to injury via paracrine stimulation of neighboring vascular cells (11, 12). It has not been determined whether the PDGF produced by endothelial cells consists of A-B heterodimers, A-A or B-B homodimers, or mixed populations of these forms, but mRNA for both A and B chains has been detected in endothelial cells (13, 14).

Previous work demonstrated that B chain expression could be enhanced in cells by transforming growth factor-β (TGF-β) (15). Recently we showed that phorbol esters, thrombin, and TGF-β enhance expression of PDGF B chain mRNA by human microvascular endothelial cells (MEC) and increase the amount of PDGF-like activity in conditioned media, and that β-adrenergic stimulation decreases the level of B chain mRNA (13, 16). Regulation of the expression of the PDGF A chain gene has not been shown previously. We now report that phorbol esters, thrombin, and TGF-β increase the level of A chain mRNA in microvascular endothelial cells, and agents that elevate cAMP cause a reduction in A chain mRNA. While these changes in A chain mRNA are qualitatively similar to the changes in B chain mRNA in microvascular endothelial cells, there are some striking quantitative differences in the regulation of the expression of these two genes. These differences suggest that endothelial cells may react to various stimuli by producing a variety of homodimeric or heterodimeric forms of PDGF.

MATERIALS AND METHODS

Human microvascular endothelial cells were obtained as previously described (17) and were grown to confluence in gelatin-coated flasks in M199 Earle’s solution supplemented with 15% fetal calf serum, endothelial cell growth factor (25 μg/ml, Collaborative Research), and heparin (10 units/ml). Cells were made quiescent by rinsing cells with M199, and then replacing media with serum-free M199 containing insulin (1 μg/ml), transferrin (5 μg/ml), and bovine albumin (0.5 μg/ml) for 20 h. Total cellular RNA was isolated by guanidinium isothiocyanate lysis and ultracentrifugation over a cesium chloride cushion as described (18). Total RNA was quantitated by absorbance at 260 nm. PDGF A and B chain and β2-microglobulin mRNA were measured using antisense 32P-labeled RNA probes generated by in vitro transcription in the presence of labeled UTP, β2-Microglobulin, and B chain probes were generated as described (16). PDGF A chain probe was generated using a 218-base pairs BamHI-Sal1 fragment of PDGF A chain cDNA subcloned into the BamHI-Sal1 sites of pGEM II (1.3-kb cDNA A chain clone; gift of C. H. Heldin, Ludwig Institute-Uppsala). Gel purified 32P-labeled RNA probes were incubated in hybridization solution with 20 μg of total RNA and subjected to RNase digestion (19, 20). The protected species were separated by electrophoresis on 5% acrylamide 8 M urea gels and visualized by autoradiography as described (19, 20). For Northern blot analysis, poly(A+) mRNA was prepared by standard techniques (21). Ten μg of poly(A+) RNA was transferred to nylon membranes and cross-linked by ultraviolet light as described (22). Hybridization with 1 x 10⁶ cpm/ml of nick translated 32P-labeled probes generated from a 1.3-kb A chain cDNA clone was performed as described (22). Filters were exposed for 6 h at –70°C. Bands were quantitated by laser densitometry.

RESULTS

In order to determine whether PDGF A chain expression in MEC is regulated by agents that regulate PDGF B chain expression, quiescent MEC were stimulated with various

13-acetate; MEC, microvascular endothelial cells; kb, kilobase.
agents and PDGF A chain mRNA was measured by RNase protection assay. Stimulation of MEC with thrombin, PMA, and TGF-β, agents known to elevate PDGF B chain mRNA (15, 16), dramatically increased PDGF A chain mRNA to levels 5–25-fold above control levels (Fig. 1). In stimulating B chain mRNA levels, PMA was most efficacious, followed in decreasing order by thrombin and TGF-β. However, for A chain mRNA induction by these same agents, the order was reversed; TGF-β was most efficacious, followed in decreasing order by thrombin and PMA (Fig. 1, laser densitometry).

Studies of PDGF A chain mRNA in a variety of cell types have shown there are several distinct A chain transcripts (3, 7, 11). In order to determine whether the increase in A chain mRNA level seen by RNase protection assay involved selective induction of one or more transcript forms, Northern blot analysis of poly(A⁺) RNA from unstimulated MEC and MEC stimulated with PMA, thrombin, and TGF-β was performed. Four different A chain transcripts were seen, and all were induced by TGF-β, thrombin, and PMA (Fig. 2, PMA data not shown). Transcripts were approximately 3.0, 2.8, 2.3, and 1.5 kb in size (Fig. 2). On longer exposure (12 h), the 3.0-kb band was easily seen. Studies in our laboratory have shown that the increase in PDGF B chain mRNA stimulated by TGF-β was prolonged compared to the increase stimulated by thrombin or PMA (see Fig. 1 and Ref. 16). The enhanced expression of A chain mRNA due to TGF-β stimulation was also prolonged (peak 4 h, duration 48 h) compared to that following stimulation with thrombin or PMA (peak 4 h, duration 8 h) (Fig. 3). The differences in time course suggest that TGF-β enhances A chain expression by a mechanism different from that used by thrombin or PMA.

Treatment of MEC with forskolin, a direct activator of the catalytic subunit of adenylate cyclase, has been shown to

**Fig. 1.** Induction of PDGF A and B chain mRNA in microvascular endothelial cells. Total cellular RNA (20 μg) from quiescent MEC stimulated with phorbol myristate acetate (PMA, 100 ng/ml for 240 min), thrombin (3 units/ml for 240 min), and TGF-β (200 pm for 240 min) was assayed for PDGF A chain, B chain, and β₂-microglobulin mRNA by solution hybridization-RNase protection assay. PMA, thrombin, and TGF-β increased B chain mRNA 5–10-fold and A chain mRNA 5–25-fold. The first lane labeled P shows undigested unprotected probes for PDGF B chain (B), PDGF A chain (A), and β₂-microglobulin (β₂) in descending order. Lane P + RNase shows the same probes digested with RNase without cellular RNA protection.

**Fig. 2.** Transforming growth factor-β and thrombin induce several distinct PDGF A chain transcripts. Poly(A⁺) RNA (10 μg) from quiescent MEC stimulated with TGF-β (200 pm for 240 min) or thrombin (3 units/ml for 240 min) was subjected to Northern blot analysis using a 1.3-kb nick translated cDNA probe for PDGF A chain. Three major species (2.8, 2.3, and 1.4–1.5 kb) of A chain transcript were induced, as well as a larger transcript of 3.0–3.2 kb.

**Fig. 3.** Time course of PDGF A and B chain mRNA induction. Total cellular RNA (20 μg) from quiescent MEC was assayed for A and B chain mRNA, and autoradiography was performed. Bands were quantitated by laser densitometry. Results are shown as fold induction of A or B chain mRNA levels versus time. Results were normalized to the β₂-microglobulin signal determined for each sample. Data points represent the mean of three separate determinations except for the 24- and 48-h time points where two determinations were made.
block induction of PDGF B chain mRNA by thrombin and TGF-β (16). In order to determine whether PDGF A chain expression was regulated in the same manner by agents that elevate cAMP, MEC were treated with forskolin or isoproterenol and A and B chain mRNA were measured. Forskolin treatment decreased levels of A chain mRNA stimulated by thrombin by 60–70%, but had minimal effect (10–20% reduction) on A chain mRNA levels stimulated by TGF-β (Fig. 4). The slight effect of forskolin on TGF-β stimulation of A chain mRNA was in marked contrast to the dramatic effect of forskolin on TGF-β stimulation of B chain mRNA (Fig. 4). Similar results were seen when cAMP levels were increased by isoproterenol treatment (data not shown). These differences suggest that there are mechanistic differences between the cAMP effects on the A chain and B chain mRNAs.

**DISCUSSION**

In this study we showed that PDGF A chain gene expression is regulated by some of the same agents that regulate PDGF B chain expression in microvascular endothelial cells. However, the pattern of regulation was different. For example, among the agents that increased B chain mRNA levels, PMA was most efficacious, followed in decreasing order by thrombin and TGF-β. However, for A chain mRNA induction by these same agents, the order was reversed. Northern blot analysis revealed the presence of several different A chain transcripts; all were increased by PMA, thrombin, and TGF-β. In addition, agents that elevate cAMP, known to block induction of PDGF B chain mRNA by thrombin and TGF-β, blocked induction of PDGF A chain mRNA by thrombin but not by TGF-β. The molecular mechanisms through which PDGF A and B chain mRNA levels are regulated are not yet known. The differential effect of cAMP on induction of PDGF A chain mRNA by thrombin and TGF-β implies that these two agents may act through different mechanisms to increase PDGF A chain message. Current work in our laboratory is focused on determining whether changes in transcription or mRNA stability are involved in these regulatory events. An important issue raised by these findings is what form of PDGF is released by microvascular endothelial cells after stimulation by the agents we have studied. Previously we have shown that thrombin, PMA, and TGF-β stimulate, and that elevation of cAMP blocks, the production of PDGF-like activity by these cells (13, 16). However, specific antibodies have not been available to allow a conclusive determination of whether the released PDGF is in homodimer (A-A or B-B) or heterodimeric (A-B) form. Differential regulation of PDGF A and B chains may provide a mechanism for altering the expressed form and activity of PDGF released by endothelial cells. The A-B, A-A, and B-B forms of PDGF may differ in their stability, secretion, binding efficiency, or activity. Endothelial cells may react to various physiologic or pathologic stimuli by producing different forms of PDGF with different effects on their target cells. For example, the results of the current studies predict that TGF-β would stimulate the production of more A chain-containing forms (A-A or A-B) than phorbol ester which would favor the production of B chain containing forms (B-B or A-B). Such a predominance of A chain product has been demonstrated for some tumor cell types (3) and has been suggested for umbilical vein endothelial cells (14). Similarly, rat smooth and skeletal muscle cells secrete a PDGF-like mitogen and express A but not B chain message (7). Previous studies in our laboratory have shown that the stimulated expression of B chain is abolished when cAMP levels are elevated either by forskolin or β-adrenergic stimulation (16). The level of A chain mRNA is much less affected by cAMP when it is increased by TGF-β (Fig. 4). Thus the A and B chain message are regulated differently in this setting, again favoring the A chain form. Presumably in the presence of cAMP the mitogenic proteins produced by these cells are more likely to be A chain homodimers than B chain homodimers or A-B heterodimers. Further work will be required to elucidate the molecular mechanism of A and B chain regulation and the biologic importance of these regulatory events in vivo.

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