Regulation of Hexose Transport in Aortic Endothelial Cells by Vascular Permeability Factor and Tumor Necrosis Factor-α, but Not by Insulin*

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Vascular permeability factor (VPF) is mitogenic for bovine aortic endothelial (BAE) cells, whereas tumor necrosis factor (TNF) is cytostatic and was found to completely block the mitogenic response to VPF. In contrast to the apparently antagonistic mitogenic effects that these two factors elicit, chronic exposure of BAE cells to either VPF or TNF resulted in significant (about 3-fold) increases in the rates of hexose transport. The concentrations required for half-maximal stimulation were 2 ng/ml (40 pM) for TNF and 4 ng/ml (100 pM) for VPF. Exposure to both factors simultaneously resulted in a greater stimulation of transport (about 7-fold) than exposure to either factor alone. Northern blot analysis indicated that the amount of message for the GLUT-1/erythrocyte form of the glucose transporter was specifically increased by treat-ment with VPF (5-fold), TNF (25-fold), or to both cytokines together (35-fold). Expression of mRNAs for the insulin-sensitive muscle/adipose transporter (GLUT-4), brain/fetal skeletal muscle transporter (GLUT-3), or the hepatic transporter (GLUT-2) were not detected in either control or treated cells. Acute or chronic exposure to insulin (10^{-9} to 10^{-6} M) did not activate hexose transport in BAE cells. Thus, glucose transport in aortic endothelial cells can be up-regulated by either VPF, a growth stimulator, or by TNF, a growth inhibitor, but not by insulin. The additive effect of the two cytokines together may be important in the control of increased glucose metabolism at sites of inflammation.

The vascular endothelium is a major target for several regulatory cytokines, including vascular permeability factor (1–5) and tumor necrosis factor (6–8). Vascular permeability factor (VPF), also known as vascular endothelial growth factor (9–11), increases vascular permeability (2, 3, 5), stim-ulates endothelial cell growth and angiogenesis (4), and induces tissue factor expression by endothelial cells.2 Tumor necrosis factor-α (TNF) also has numerous effects on endothelial cells including in vitro inhibition of endothelial cell growth (12), inhibition of cell movement (14), and inhibition of angiogenesis (15). TNF promotes angiogenesis in vivo (16, 17). Endothelial cells treated with TNF are induced to synthesize interleukin 1 (18, 19) and granulocyte/macrophage colony-stimulating factor (20). Several cell surface proteins are also induced by TNF, including a neutrophil adhesion molecule (21), a lymphocyte adhesion molecule (22), and tissue factor (23). The combined effect of TNF and VPF on the induction of tissue factor in endothelial cells could lead to intravascular coagulation. It has also been suggested that the expression of VPF by certain tumors could lead to increased sensitivity to the action of TNF.2

The effects of TNF and VPF on endothelial cells appear to be similar in some instances. For example, both cytokines induce the expression of tissue factor by endothelial cells (23). In contrast, the cytokines seem to elicit opposite effects on endothelial cell growth (4, 13). In order to better understand the effects of VPF and TNF on endothelium, we have undertaken a study of particular biochemical responses of cultured endothelial cells to each of these factors individually and in combination. The present study describes the effect of VPF and TNF on glucose transport, a regulatory process central to cellular metabolism.

EXPERIMENTAL PROCEDURES

Materials—The GLUT-1 and GLUT-4 forms of the glucose transporter cDNAs were as described by Kaestner et al. (24). The GLUT-2 and GLUT-3 isoforms of the transporter cDNAs were obtained from Dr. Grahame Bell (25). VPF was purified from cultures of guinea pig line 10 tumor cells as previously described (4, 5). The anti-VPF antiserum and protocol for immunoadsorption was as previously described (4). Recombinant TNF was the generous gift of Biogen Inc., Cambridge, MA. Molar concentrations were calculated using M, = 40,000 for VPF, and M, = 51,000 for TNF trimer.

Cell Culture—Clone JVO17A of bovine aortic endothelial cells (26) were the generous gift of Dr. G. Olander, Monsanto Co., and were grown in DMEM plus 10% calf serum. For glucose transport experiments, confluent cells were re-fed 4 days post-confluence with DMEM supplemented only with 0.5% growth factor free bovine serum albumin. Twenty-four hours later the cells were re-fed with the same mixture and exposed to the proper agent (insulin, VPF, or TNF). Cell growth experiments were performed in 96-well plates in DMEM plus 10% calf serum using an acid phosphatase assay to determine cell number, as described previously (27).

2-Deoxyglucose Transport—The assay was performed as described previously (28). Briefly, the glucose transport assay was carried out in a volume of 1 ml of Krebs-Ringer/Hepes buffer, pH 7.4, 0.1% bovine serum albumin in a 3.5-cm dish for 3 min at 37 °C, containing 100 μM 2-deoxyglucose and 1 μCi of [3H]2-deoxyglucose. After terminating the transport assay with three washes of ice-cold assay buffer, each monolayer was solubilized in 1 ml of a buffered digitonin solution (29). A 0.3-ml aliquot was removed for determination of radioactivity by liquid scintillation counting, and two 0.1-ml aliquots were used for measurement of protein according to Lowry et al. (30). Under these conditions hexose uptake was linear for at least 15 min, and at least 90% of the 2-deoxyglucose was phosphorylated at the termination of the assay as determined through the use of the Somogyi reagent (31). Measurements were made in triplicate and cor-

1 Clauss, M., Gerlich, M., Gerlich, H., Brett, J., Wang, F., Famili-letti, P. C., Pan, Y.-E., Olander, J. V., Connolly, D. T., and Stern D. (1990) J. Exp. Med., in press.

2 Clauss, M., Gerlich, M., Gerlich, H., Brett, J., Wang, F., Famili-letti, P. C., Pan, Y.-E., Olander, J. V., Connolly, D. T., and Stern D. (1990) J. Exp. Med., in press.
TNF; 2.5 nM was maximally stimulated about 3-fold by chronic incubation to demonstrate that the rate of glucose transport in BAE cells was not affected by TNF alone. The effect was additive when the two agents were combined, and the concentration of TNF required for half-maximal activation of glucose transport was 2 nM (40 nM TNF, 2.5 nM VPF) for 17 h resulted in enhanced accumulation of GLUT-1 mRNA (Fig. 4). Densitometric measurement of the autoradiograms indicated approximately 25-fold (TNF) and 5-fold (VPF) increases in the rate of hexose transport above basal level. When the two cytokines were added together but not treated with anti-VPF antisera, the cells were assayed for their ability to transport [3H]2-deoxyglucose.

**RESULTS**

**TNF Blocks the Mitogenic Response of BAE Cells to VPF**

TNF has been previously shown to block the mitogenic effects of basic fibroblast growth factor (17). Fig. 1 shows that TNF can also completely block the VPF-stimulated mitogenic response of BAE cells. When added alone, VPF stimulated BAE cell growth 3.5-fold relative to untreated control cultures, but addition of 10 ng/ml TNF completely prevented the stimulation by VPF. The concentration of TNF required for half-maximal inhibition of BAE cell growth was about 1 nM (20 pm). TNF alone did not significantly reduce the cell number below the numbers observed in control cultures, nor did it affect the cell viability even at the highest concentrations tested.

**VPF and TNF Stimulate Glucose Transport in BAE Cells**

In preliminary experiments it was determined that TNF and VPF both stimulated glucose transport and that the concentrations necessary for half-maximal activation of glucose transport were 2 ng/ml (40 pm) and 4 ng/ml (100 pm) for TNF and VPF, respectively. Maximal stimulation of glucose transport was obtained when the cells were exposed to saturating concentrations of the two agents (16.5 ng/ml, 0.35 nM TNF; 2.5 nM VPF) for 12-20 hr. The data displayed in Fig. 2 demonstrate that the rate of glucose transport in BAE cells was maximally stimulated about 3-fold by chronic incubation (17 h) with saturating concentrations of either VPF or TNF. The effect was additive when the two agents were combined, resulting in a 7-fold increase in the rate of transport. Immunoadsorption using IgG specific for VPF blocked the additive effect, whereas nonimmune serum had no effect.

**Effect of Insulin on Glucose Transport in BAE Cells**

Acute exposure (30 min) of the BAE cells to concentrations of insulin ranging from 10^-9 to 10^-6 M did not result in stimulation of hexose uptake above basal levels (Fig. 3). In addition, chronic exposure of the cells to 10^-7 M insulin for periods of up to 24 h did not alter the rate of hexose entry into the cell (data not shown).

**VFP and TNF Induce GLUT-1-type Glucose Transporter mRNA in BAE Cells**

BAE cell mRNA was analyzed by Northern blot analysis using specific CDNA probes for the various isoforms of glucose transporter. Preliminary experiments indicated that accumulation of GLUT-1 mRNA after exposure of the cells to the agents was delayed, with maximal accumulation occurring between 12-20 h after exposure to either TNF or VPF. The kinetics of the increase in specific message levels correspond well with the increase in transporter rates measured above. Exposure of the BAE cells to saturating levels of either VPF (2.5 nM) or TNF (0.35 nM) for 17 h resulted in enhanced accumulation of GLUT-1 mRNA (Fig. 4). Densitometric measurement of the autoradiogram indicated approximately 25-fold (TNF) and 5-fold (VFP) accumulations of message above basal level. When the two cytokines were added together, a 35-fold enhancement above basal level was obtained (Fig. 4). These levels are...
significantly higher than the increases in glucose transport activity, and may indicate that post-transcriptional or post-translational mechanisms are regulating this process.

After analysis for GLUT-1, the filter was stripped of hybridized probe and re-probed sequentially for GLUT-2, -3, -4, and actin. No hybridization signal was observed for any of the other isoforms of the glucose transporter, even though 49-h exposures of the autoradiographic films were used instead of the overnight exposure used for detection of GLUT-1. It is estimated that message for the other isootypes would have been detectable if present in levels approximating 10% of GLUT-1. Actin mRNA was detectable in overnight exposure indicating that intact message remained on the blot.

**DISCUSSION**

TNF completely blocked the mitogenic response of BAE cells to VPF. This is consistent with previous observations in which TNF blocked the mitogenic response of endothelial cells to basic fibroblast growth factor, another endothelial cell growth factor (17). TNF is thus a general physiological antagonist toward endothelial cell growth factors.

In contrast, BAE cell glucose transport rates were stimulated by both cytokines, and the observed effects were additive rather than antagonistic. Furthermore, the BAE cells were completely nonresponsive to insulin under conditions in which muscle or adipose would rapidly increase glucose transport rates. Glucose transport is under the control of a family of related transporters, the expression of which are tissue specific and developmentally regulated (34). We therefore used Northern blot analysis to probe BAE cell mRNA for the presence of each of four isoforms of glucose transporter. Only the erythrocyte/GLUT-1 type of transporter mRNA was detected by this method. Since the GLUT-1 transporter is known to be only minimally insulin-responsive, this result is consistent with the failure of insulin to stimulate transport in these cells. However, both VPF and TNF were found to induce GLUT-1 transporter mRNA, and not mRNA of the other transporter isoforms. The message level of GLUT-1 mRNA increased about 35-fold in the presence of maximal doses of both cytokines together.

Why is GLUT-1 under the control of VPF and TNF? The antagonistic mitogenic effect of the two factors makes it unlikely that GLUT-1 induction and the subsequent increase in glucose transport are related to the mitogenic response per se. In fact, studies in other systems indicate that the expression of this glucose transport gene is not strictly associated with a particular phase of the cell cycle and is dissociable from DNA synthesis since fibroblast growth factor and epidermal growth factor were shown to stimulate GLUT-1 mRNA accumulation with no effect on DNA synthesis (12). Likewise, the lack of insulin responsiveness of the BAE cells makes it unlikely that these effects are related to systemic glucose control. We propose, instead, that the induction of GLUT-1 in endothelial cells by VPF and TNF is part of a general pleiotropic cellular response to inflammatory cytokines. Sites of inflammation are often necrotic and would be expected to require enhanced glucose metabolism. This view is supported by the recent observation that TNF and VPF can act together in the induction of tissue factor on endothelial cells. Tissue factor expression by endothelial cells transforms the endothelium from a nonthrombogenic surface to a procoagulant surface, and is thought to be a specific manifestation of the inflammatory response. The present data suggest that the increased glucose transport in response to TNF and VPF may also be a part of this process and may be a necessary component in the activation of the endothelium during inflammation.

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