Stimulation of adhesion molecule expression in human endothelial cells (HUVEC) by adrenomedullin and corticotrophin

Eleni Hagi-Pavli, Paula M. Farthing, and Supriya Kapas

Molecular Signalling Group, Clinical Science Research Centre, Barts & the London, Queen Mary, University of London, London E1 2AD, United Kingdom

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Hagi-Pavli, Eleni, Paula M. Farthing, and Supriya Kapas. Stimulation of adhesion molecule expression in human endothelial cells (HUVEC) by adrenomedullin and corticotrophin. Am J Physiol Cell Physiol 286: C239–C246, 2004. First published October 8, 2003; 10.1152/ajpcell.00036.2003.—Adrenomedullin (AM) and corticotrophin (ACTH) are both vasoactive peptides produced by a variety of cell types, including endothelial cells. Although AM and ACTH are considered to be important in the control of blood pressure and the response to stress, respectively, their role in inflammation and the immune response has not been clarified. This study shows, with the use of a cell-based ELISA, that AM and ACTH induce cell surface expression of the adhesion molecules E-selectin, VCAM-1, and ICAM-1 on human umbilical vein endothelial cells (HUVEC). Furthermore, this effect appears to be mediated in part via elevation of cAMP, given that both peptides elevate cAMP, the cell-permeable cAMP analog dibutyryl cAMP is able to mimic induction of all three cell adhesion molecules and the effect of AM and ACTH is inhibited by the adenyl cyclase inhibitor SQ-22536. These findings demonstrate a role for AM and ACTH in the regulation of the immune and inflammatory response.

E-selectin; intercellular adhesion molecule-1; vascular cell adhesion molecule-1; adrenomedullin; adrenocorticotropic hormone; human umbilical vein endothelial cells

THE ENDOTHELIAL LINING is an ever-changing interface that responds to a variety of stimuli originating from the circulating blood as well as neighboring cells and tissues. It thereby has an active role in the physiological adaptation or pathophysiological dysfunction of a given region of the vasculature (22). The endothelium is ideally designed for its intended purpose given its exclusive anatomical position between blood and tissues as well as its ability to produce a vast range of humoral factors, such as cytokines, growth stimulators and inhibitors, pro- and anticoagulants, nitric oxide, and vasoactive peptides.

Modulation of the vascular endothelium by cytokines has been extensively studied at the cellular, molecular, and genetic regulatory levels. Three cell surface adhesion molecules have been well defined: E-selectin (4, 13), ICAM-1 (34), and VCAM-1 (41). E-selectin is a member of the selectin family of adhesion molecules and is expressed primarily by endothelial cells, although there have been reports that it is expressed by human gingival epithelial cells (43). It plays a critical role in mediating the initial step of leukocyte migration from peripheral blood into tissues (59, 60) by binding to sialylated oligosaccharides (sialyl Lewis X) expressed by neutrophils and monocytes and promoting loose adhesion. It is also important in mediating the selective migration of a subset of peripheral blood memory T lymphocytes, which express a form of sialyl Lewis X known as cutaneous lymphocyte-associated antigen into skin and oral mucosa. Although E-selectin is expressed at low levels by endothelial cells in skin and oral mucosa, levels are upregulated in inflammatory disease (21), and it is induced on endothelial cells in vitro by IL-1β, TNF-α, LPS (5), thrombin (50), IL-3 (9), and phorbol esters (36).

VCAM-1 and ICAM-1 are both members of the immunoglobulin superfamily and are expressed on endothelial cells as well as a variety of other cell types (48, 53). ICAM-1 is a ligand for the two integrin molecules, LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18) (15), and mediates the firm attachment of all classes of leukocytes to endothelium (33). Although constitutively expressed at low levels, it is upregulated in disease (25) and induced in vitro by the cytokines IL-1β, TNF-α, interferon (INF)-γ, and PMA on many cell types (17, 49, 54). In contrast, VCAM-1 is usually absent from normal vascular endothelium but is induced in vitro on endothelial cells after stimulation with IL-1, IL-4, or TNF-α (61). However, VCAM-1 is expressed on vascular endothelium of post-capillary venules in a variety of pathological conditions, including delayed hypersensitivity and allograft rejection (8, 48), and also plays an important role in promoting firm adhesion of lymphocytes, monocytes, basophils, and eosinophils to the vessel wall through interactions with the leukocyte integrin molecule α4β7 (also known as VLA-4) (16, 19, 41). In vitro studies (39, 40) have shown different roles of ICAM-1 and VCAM-1 in lymphocyte adhesion and transmigration across activated endothelial cell monolayers. These studies propose that VCAM-1 mediates the initial adhesion of T cells and monocytes to activated endothelial cells and that ICAM-1 may be important in reinforcing their adhesion and facilitating the cells’ subsequent transmigration.

Adrenomedullin (AM) is a multifunctional peptide expressed and secreted from a wide variety of cells and tissues (23). It is noted primarily for its role in the vasculature and maintaining blood pressure (38, 52), but recently it has been implicated in the progression of trauma, infection, and sepsis (10, 20). Studies from our laboratory (1, 32) and by others (35, 44) strongly suggest a role for AM in the inflammatory response as well as in mucosal defense.

Corticotropin (ACTH) is well documented to be a stress-induced hormone that stimulates the synthesis and secretion of glucocorticoids and, as such, is essential for life. It is produced primarily by the pituitary gland, but recently it has become...
clear that production is more widespread and that it is produced by a variety of cells, including keratinocytes (30). ACTH stimulates the release of glucocorticoids, and their anti-inflammatory activity is well documented, but whether ACTH itself plays an immunomodulatory role has not been studied.

The aim of this study was to investigate whether AM and ACTH are immunostimulatory and induce adhesion molecules important in leucocyte migration on the surface of endothelial cells. We report that AM and ACTH significantly induce cell surface expression of E-selectin, VCAM-1, and ICAM-1 on human umbilical vein endothelial cells (HUVEC) and, in addition, that this expression appears to be mediated in part via cAMP.

EXPERIMENTAL PROCEDURES

Reagents. Human AM was obtained from Phoenix Pharmaceuticals (Belmont, CA). ACTH (Synacthen) was from Ciba Laboratories (Horsum, UK). Tissue culture materials were from Life Technologies (Paisley, Scotland). [3H]cAMP was from Amersham Pharmacia (Belmont, CA). ACTH (Synacthen) was from Ciba Laboratories (Poole, UK). Monoclonal anti-human ICAM-1 antibody was purchased from Serotec (Oxford, UK). Monoclonal anti-human E-selectin (clone BBIG-E4), monoclonal anti-human VCAM-1, and TNF-α antibodies were purchased from R&D Systems Europe (Oxon, UK), and NF-κB antibody was from Santa Cruz Biotechnology (Santa Cruz, CA). All other reagents were the best grade available.

Cell culture. HUVEC were obtained from BioWhittaker UK (Wokingham, UK) and maintained by following the supplier’s instructions. Cells were used from passages 2 to 4 in all experiments.

E-selectin, VCAM-1, and ICAM-1 ELISA. The assay for E-selectin, VCAM-1, or ICAM-1 expression on the surface of HUVEC was performed by an ELISA after cell fixation. Cells (105 cells/plate) were seeded and grown in 96-well flat-bottomed microtiter plates in 200 μl of serum-free medium. After 24 h, the medium was replaced with fresh medium containing the various peptides to be tested. Plates were incubated with 1) varying concentrations of peptide (AM, ACTH, or TNF-α), 2) peptide alone (10−7 M), 3) peptide (10−7 M) and inhibitor SQ-22536 (10−6 M), 4) SQ-22536 alone (10−6 M), or 5) DBcAMP alone (10−6 M) for either 4 or 16 h. At the end of this incubation, the cells were washed with HBSS and incubated with E-selectin, VCAM-1, or ICAM-1 antibody at 37°C in a humidified atmosphere of 5% CO2. At the end of the incubation period, cells were washed with HBSS and then fixed with 2% paraformaldehyde dissolved in PBS (pH 7.5). After being washed with HBSS, cells were incubated for 1 h at 37°C with 1% BSA in HBSS. Cells were then washed and incubated at room temperature for 30 min with biotinylated rabbit anti-mouse IgG, followed by the addition of a preformed avidin-biotinylated horseradish peroxidase complex. Peroxidase activity was viewed under UV illumination. Oligonucleotide primers used for E-selectin were 5′-CTCTGACAGAAGAGCCAAAG-3′ (sense) and 5′-ACTTGAAGCTTCAAGGCCAGG-3′ (antisense) to amplify a 255-bp PCR product. Primers for VCAM-1 were 5′-GGATTCTCTGCCCACAGTA-3′ and 5′-CCTGGCTCAACGATCTA-3′ (antisense) to amplify a 724-bp PCR product (primer annealing at 60°C). Primers for ICAM-1 were 5′-GGCAAGACCTTACCTAATA-3′ (sense) and 5′-CATCAGCTGCACCTGTG-3′ (antisense) to amplify a 646-bp PCR product with an annealing temperature of 62°C.

Preparation of subcellular proteins and Western blotting. Cells were homogenized in a lysis buffer containing 50 mM NaCl, 25 mM Tris-Cl (pH 8.1), 0.5% Nonidet P-40, 0.5% sodium deoxycholate, 1 mM PMSF, 10 μg/ml leupeptin, 10 μg/ml pepstatin, and 25 μg/ml aprotinin. Nuclei were separated from the cytoplasm by centrifugation at 2,500 rpm at 4°C for 5 min, washed briefly with lysis buffer without Nonidet P-40, resuspended in nuclear extraction buffer (20 mM Tris-HCl, pH 8.0, 20 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, and 25% glycerol), and lysed by the addition of NaCl to a final concentration of 400 mM. The suspension was kept on ice for 10 min, vortexed, and then centrifuged for 10 min (14,000 rpm). The supernatant contained the nuclear protein extract. The supernatant of the first centrifugation step, which contained the cytoplasmic fraction, was centrifuged at 14,000 rpm, and the resulting supernatant was used for Western blot analysis.

Cytoplasmic or nuclear protein (50 μg) was heated to 99°C for 4 min, loaded into sample wells, resolved on a 12% tricine SDS-polyacrylamide gel (Novex, San Diego, CA), and run at 120 V for 2 h. Transfer blotting was accomplished by using the same apparatus, and proteins were transferred to a polyvinylidene difluoride Immobilon-P membrane (Millipore, Watford, UK) at 30 V for 1 h. Membranes were blocked overnight at 4°C in a solution of 5% dried milk in PBS containing 0.1% Tween 20. Membranes were then washed and incubated for 60 min at room temperature in a 1:1,000 dilution of rabbit anti-NF-κB p65 polyclonal antibody (Santa Cruz Biotechnology), followed by a three-time wash in PBS and incubation in 1:200 goat anti-rabbit IgG (Vector Laboratories, Peterborough, UK) for 60 min at room temperature. Membranes were washed three times in PBS, and the signal was amplified/detected by using the ECL Plus kit following the manufacturer’s instructions (Amersham plc, Little Chalfont, UK).

Measurement of cAMP. Cells were washed twice with PBS and incubated in media with increasing concentrations of either AM or ACTH for 4 h in the presence of 50 μM IBMX. Media and cells were then removed, boiled for 5 min, and centrifuged for 5 min, and the supernatant was stored at −20°C until required for assay. Extracellular and intracellular cAMP content was measured using an in-house specific binding protein assay (31).

Statistical analysis. Arithmetic means and standard deviations were calculated. One-way analysis of variance was used to test whether factors had an effect on basal (control) levels and expression of E-selectin, VCAM-1, or ICAM-1. Dennett’s test was used to test whether SQ-22536, the adenylyl cyclase inhibitor, had any effect or stimulated adhesion molecule expression on HUVEC.
concentration for threshold stimulation was $10^{-12}$ M, and maximal levels were reached at $10^{-7}$ M. Threshold stimulation occurred at $10^{-13}$ M ACTH and was maximal at $10^{-7}$ M.

Cell surface expression of adhesion molecules with time. Figure 2, A–C, shows the effects of $10^{-7}$ M AM or ACTH on adhesion molecule expression when HUVEC were exposed to either peptide for 4 or 16 h. Because it is well documented that AM (23) and ACTH (14) elevate cAMP, HUVEC were exposed to the cell-permeable cAMP mimic DBcAMP. In these experiments TNF-α (5 ng/ml), a potent activator of adhesion molecule expression, was used as a positive control.

Figure 2A shows that both AM and ACTH caused maximal increases in E-selectin expression after 4 h of incubation and that levels reduced significantly by 16 h, although they did not reach basal levels. This pattern of induction was similar to that of TNF-α, but interestingly, levels of induction were greater with the peptides. DBcAMP also caused a significant induction in E-selectin at both 4- and 16-h stimulation times (Fig. 2A), and, like TNF-α, AM, and ACTH, the highest levels were found at 4 h.

VCAM-1 expression was significantly upregulated at 4 h when HUVEC were incubated with AM or ACTH ($10^{-7}$ M) and remained at this level after 16 h (Fig. 2B). Compared with 4 h, a decrease in VCAM-1 expression occurred after 16 h of stimulation with TNF-α. Incubation of HUVEC with DBcAMP caused a significant induction of VCAM-1 expression that was higher at 16-h than at 4-h stimulation times.

ICAM-1 was also induced on the surface of HUVEC by AM or ACTH at both 4- and 16-h incubation times (Fig. 2C). Induction of ICAM-1 expression was higher at 16 h than at 4 h of stimulation, but, in contrast, levels of ICAM-1 induced by TNF-α did not vary between 4 and 16 h of stimulation. The cell-permeable cAMP mimic DBcAMP also induced ICAM-1 expression on HUVEC, but not to the same levels achieved by either AM or ACTH.

Effect of differing concentrations of AM or ACTH on intracellular cAMP levels. To determine whether the effects of AM and ACTH were mediated by cAMP, we determined levels after incubation of HUVEC for 4 h with increasing concentrations of peptides. Figure 3 shows that levels of cAMP increased significantly with $10^{-12}$ M ACTH and reached maximal levels at $10^{-9}$ M ACTH. Induction of cAMP was also observed with $10^{-9}$ and $10^{-7}$ M AM. No increase in cAMP was seen after incubation with TNF-α (results not shown).

Effect of adenyl cyclase inhibition on induced cell surface expression of adhesion molecules. Figure 4, A–C, shows the effect of the adenyl cyclase inhibitor SQ-22536 on AM- and ACTH-induced adhesion molecule expression. Figure 4A shows that SQ-22536 significantly inhibited HUVEC cell surface expression of E-selectin in response to both AM and ACTH. Similar observations were made with respect to stimulated ICAM-1 and VCAM-1 expression (Fig. 4, B and C, respectively). However, in all cases, levels of adhesion molecules were always greater than at baseline. Figure 4, A–C, also shows that both AM and ACTH induced E-selectin, ICAM-1, and VCAM-1 mRNA expression in HUVEC and that mRNA expression was inhibited by the adenyl cyclase inhibitor.

Nuclear translocation of NF-κB. Incubation of HUVEC with AM or ACTH resulted in activation of the transcription factor NF-κB (Fig. 5). When activated, NF-κB translocates...
Fig. 2. Expression of cell surface E-selectin (A), VCAM-1 (B), or ICAM-1 (C) on HUVEC in the presence of $10^{-7}$ M ACTH, AM, or dibutryl cAMP (DBcAMP) or 5 ng/ml TNF-α for 16 h (open bars) and $10^{-7}$ M ACTH, AM, or DBcAMP or 5 ng/ml TNF-α for 4 h (hatched bars). Values are means ± SE, n = 4, ***$P < 0.001$ compared with basal levels of expression (analysis of variance). β1 refers to the β1-integrin, which served as a positive control for adhesion molecule expression.
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from the cytoplasm to the nucleus after proteolysis, ubiquitination, and degradation of the inhibitor protein IkB.

DISCUSSION

This is the first report to show that the vasoactive peptides AM and ACTH induce E-selectin, VCAM-1, and ICAM-1 expression on HUVEC and that their induction by AM or ACTH appears to be dependent, at least in part, on elevation of cAMP. These findings suggest that AM and ACTH may play an important role in the immune and inflammatory response by inducing adhesion molecules on the surface of endothelial cells and regulating leukocyte recruitment from peripheral blood into the tissues.

To our knowledge there has been only one other report to date (55) to show that a vasoactive peptide may play a role in the regulation of leukocyte adhesion to the endothelium. Calcitonin gene-related peptide (CGRP), which shares modest structural homology with AM, has been shown to upregulate the adhesiveness of HUVEC for leukocytes in a dose-dependent manner (55), although the mechanism was not investigated. One explanation is that CGRP induces adhesion molecule expression on HUVEC, and this is consistent with our findings that AM induces E-selectin, VCAM-1, and ICAM-1 on endothelial cells.

In recent years several studies have examined the role of cAMP in the regulation of adhesion molecule expression, but the findings have been conflicting. The literature may be divided into 1) those studies that have looked at the effect of cAMP on endothelial cells and their adhesiveness toward leukocytes and 2) those studies that have examined the effect of cAMP-elevating agents on adhesion molecule expression.

CAMP has been shown to inhibit adherence of polymorphonuclear leukocytes to nylon fibers (6, 7) but to increase their adherence to porcine endothelial cells (42). Others have shown that agents that increase cAMP levels in endothelial cells promote the adherence of monocytes and lymphocytes (55, 57). Turunen et al. (57) found that the cytokine IL-1 induced lymphocyte migration through rat endothelial cell monolayers and that this effect was mimicked by forskolin and DBcAMP. Furthermore, IL-1 induced cAMP with peak levels after 15 min, and this effect was reduced by the adenylyl cyclase inhibitor dideoxyadenosine (56, 57). Similarly, Sung et al. (56) showed that HUVEC stimulated with epinephrine, norepinephrine, isoproterenol, cholera toxin, forskolin, or DBcAMP showed an increase in adhesion for U937 cells (a human monocyte cell line), and this correlated directly with an increase in cAMP levels. Although none of the researchers examined adhesion molecule expression, one possible explanation is that the increase in leukocyte adhesion to endothelial cells is due to induction of cell surface adhesion molecule expression. These
findings are in agreement with our observations that AM and ACTH, which elevate cAMP, also induce high levels of E-selectin, VCAM-1, and ICAM-1.

Other studies have examined the role of cAMP on adhesion molecule expression by HUVEC and found that cAMP has no effect on constitutive or TNF-α-stimulated ICAM-1 expression (37, 47). In addition, Pober et al. (47) found that neither forskolin nor IBMX, nor both in combination, induced E-selectin expression on endothelial cells but that each independently inhibited TNF-α-induced E-selectin and VCAM-1 expression. The experimental conditions in these studies on ICAM-1 expression were somewhat different from those used in our experiments. Both authors employed pharmacological agents to elevate cAMP in HUVEC, after which they stimulated cells with TNF-α and then looked at the effect on ICAM-1 expression. In contrast, we have exposed HUVEC to peptides that cause an elevation in cAMP and then measured cell surface adhesion molecule expression. Our observations that AM and ACTH elevate cAMP, that both peptides induce E-selectin, VCAM-1, or ICAM-1 on HUVEC, and that this induction is mimicked by DBcAMP but prevented by the adenyl cyclase inhibitor SQ-22536 suggest that cAMP plays an important role in the regulation of cell surface expression of E-selectin, VCAM-1, and ICAM-1 by HUVEC. However, our results also suggest that AM and ACTH may regulate adhesion molecule expression by a cAMP-independent mechanism. Low doses of AM (between 10^-12 and 10^-10 M) and 10^-13 M ACTH significantly increase adhesion molecule expression without increasing cAMP, and the cAMP inhibitor SQ-22536 does not abolish adhesion molecule expression in response to ACTH and AM entirely. Thus it seems likely that there are multiple pathways regulating adhesion molecule expression by HUVEC. This is also suggested by our observations and those of others that TNF-α does not elevate or else causes only a very small elevation in cAMP (47), and its effects on E-selectin, VCAM-1, and ICAM-1 expression by HUVEC are not prevented by the adenyl cyclase inhibitor SQ-22536.

E-selectin is reported to appear within 1–2 h after cytokine treatment and to reach maximal levels between 4 and 6 h, after which expression decays rapidly in the continued presence of cytokine until it finally reaches basal levels by 24–48 h (45). This pattern of expression is consistent with the role of E-selectin in the early stages of leukocyte adhesion to the endothelium before migration into the tissues. Our results with TNF-α confirmed this pattern of induction, which was similar to that seen with AM, ACTH, and the cAMP mimic DBcAMP. This suggests that although there may be multiple signaling pathways regulating induction of E-selectin, there may be a later common pathway, and it is likely that NF-κB plays a role.

NF-κB is a nuclear transcription factor that has been shown to play an important role in the regulation of the immune response, among many other functions. Five mammalian NF-κB family members have been identified, and these all share a highly conserved Rel homology domain responsible for DNA binding, dimerization, and binding with the inhibitor IκB (12). In resting cells NF-κB lies within the cytoplasm in an inactive form in association with its inhibitor, IκB. After activation of the cell, which can take place in response to a number of stimuli, including cytokines and viral or bacterial products, intracellular signals result in degradation of IκB and activation of NF-κB, which then translocates to the nucleus (3). This results in activation of transcription of a number of early response genes, including adhesion molecules and cytokines. We have shown that incubation of HUVEC with AM or ACTH results in translocation of NF-κB from the cytoplasm to the nucleus. Thus, in addition to the elevation of cAMP, it seems likely part of the mode of action of AM in inducing adhesion molecule expression involves the transcription factor NF-κB. The intervening pathways between cAMP induction and NF-κB translocation remain to be elucidated.

VCAM-1 mediates firm adhesion of leukocytes to endothelium subsequent to the weak adhesion mediated by selectins and is also important in transendothelial cell migration. Consistent with this, VCAM-1 expression is observed within 4–6 h after cytokine treatment and reaches its peak levels within 12–18 h (61). A similar pattern of expression was seen in our studies with AM and ACTH, suggesting that both may play a physiological role in VCAM-1 induction and thus leukocyte adhesion.

ICAM-1 expression by endothelial cells has been reported in response to various stimuli, including IL-1, IL-4, TNF-α, retinoic acid, and IFN-γ (54). ICAM-1 expression reaches peak levels by 18–24 h and remains high as long as there is active cytokine present (46). Our results with AM and ACTH show that ICAM-1 levels were high at 4 h and slightly increased at 16 h. These findings are consistent with the pattern described above and also with a role for ICAM-1 in mediating firm adhesion of leukocytes to endothelium.

AM is a ubiquitous multifunctional peptide and is a product of vascular endothelial cells (26), as well as a number of other cell types. AM production by endothelial cells is increased by LPS, and high levels have been observed in septic shock (18, 24) and systemic inflammatory response syndrome, where they appear to correlate with disease severity (58). It has been proposed that AM plays a key role in the inflammatory response, but the mechanisms underlying this are not clear and there is evidence that AM may both upregulate and downregulate localized and systemic inflammatory responses (20). This complexity is reflected in the results of our study, which show induction of adhesion molecule expression on endothelial cells by AM and which appear to conflict with those of Shindo et al. (51), who showed that transgenic mice that overexpress AM show greater resistance to LPS-induced shock than wild-type control mice. Liver damage was minimal and, interestingly, the number of neutrophils present in the livers of transgenic mice was significantly lower than in controls. Because neutrophils use adhesion molecules to bind to endothelium before accessing the tissues and we have shown that AM induces adhesion molecules, it might be expected that transgenic mice would show increased numbers of neutrophils in the liver. However, the findings of the two studies are not directly comparable, because our observations were carried out in vitro and those of...
Shindo et al. were done in vivo. Furthermore, it is likely that AM has multiple effects on different tissues and may provide protection against LPS-induced shock in several different ways.

There is evidence that AM has an immunoprotective role against microorganisms. We have shown previously that AM is present in body secretions and possesses antimicrobial activity against Gram-positive and -negative members of the skin, oral, respiratory tract, and gut microflora (1, 29). This, together with our findings that AM induces E-selectin, VCAM-1, and ICAM-1 on HUVEC, which aids the recruitment of leukocytes able to resist ingress of microorganisms, provides further supporting evidence for this concept.

ACTH is produced in response to stress by the pituitary gland, from where it is released into the circulation and stimulates release of glucocorticoids from the adrenal cortex. ACTH release and glucocorticoids have been shown to be immunosuppressive; however, very little is known about the effects of ACTH itself on the immune response. ACTH has been reported to reduce immunoglobulin production in lymphocytes (11) as well as to increase growth and differentiation (2). These responses may be receptor-mediated, because ACTH receptors have been found to be present on lymphocytes (27, 28). Our results suggest that ACTH may have an immunoregulatory role and promote migration of leukocytes through endothelium by inducing adhesion molecule expression. These findings are extremely interesting because the effects of ACTH appear antagonistic to those of glucocorticoids, whose release they stimulate and which are immunosuppressive. Presumably, the effects of ACTH in vivo will depend on the balance between the levels of ACTH and glucocorticoids as well as other stimulatory and inhibitory factors.

In summary, we have shown that AM and ACTH upregulate human endothelial cell surface expression of E-selectin, VCAM-1, and ICAM-1 on human endothelial cells and that this induction is mediated via cAMP and NF-κB. These observations suggest that both peptides play an important role in regulation of the inflammatory and immune response by inducing adhesion molecule expression on human endothelial cells and promoting migration of leukocytes from peripheral blood into the tissues. Studies are presently under way to elucidate the mechanisms by which these peptides regulate adhesion molecule transcription and to study their role in vitro.

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