Effect of Factor XIII on Endothelial Barrier Function

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Summary

The effect of factor XIII on endothelial barrier function was studied in a model of cultured monolayers of porcine aortic endothelial cells and saline-perfused rat hearts. The thrombin-activated plasma factor XIII (1 U/ml) reduced albumin permeability of endothelial monolayers within 20 min by 30 ± 7% (basal value of 5.9 ± 0.4 × 10^{-6} cm/s), whereas the nonactivated plasma factor XIII had no effect. Reduction of permeability to the same extent, i.e., by 34 ± 9% could be obtained with the thrombin-activated A subunit of factor XIII (1 U/ml), whereas the iodoacetamide-inactivated A subunit as well as the B subunit had no effect on permeability. Endothelial monolayers exposed to the activated factor XIII A exhibited immunoreactive deposition of itself at interfaces of adjacent cells; however, these were not found on exposure to nonactivated factor XIII A or factor XIII B. Hyperpermeability induced by metabolic inhibition (1 mM potassium cyanide plus 1 mM 2-deoxy-d-glucose) was prevented in the presence of the activated factor XIII A. Likewise, the increase in myocardial water content in ischemic-reperfused rat hearts was prevented in its presence. This study shows that activated factor XIII reduces endothelial permeability. It can prevent the loss of endothelial barrier function under conditions of energy depletion. Its effect seems related to a modification of the paracellular passageways in endothelial monolayers.

Key words: edema • endothelial permeability • heart • ischemia-reperfusion • recombinant human factor XIII

The endothelium forms a barrier for solutes and macromolecules between the luminal and interstitial space. Under pathophysiologic conditions, loss of endothelial barrier function is predominantly due to an increase in paracellular permeability leading to enhanced extravasation of macromolecules and fluid. The resulting extracellular edema can compromise the function or may even jeopardize survival of the affected organ.

Factor XIII is a transglutaminase (endo-γ-glutamine: ϵ-lysine transferase, EC 2.3.2.13) that catalyzes the formation of γ-glutamyl-ϵ-lysyl cross-links between adjacent polypeptide chains. It plays an important role in the course of coagulation and fibrinolysis (for reviews, see references 1 and 2). The plasma proenzyme is a heterotetramer consisting of two types of subunits (A and B, with molecular masses of ~83 and 77 kD, respectively) which are noncovalently associated. The plasma factor XIII is activated by thrombin-mediated cleavage of an NH_2-terminal peptide from the A subunits which then become the active transglutaminases. The function of the B subunits is not fully understood at present. It seems to protect the A subunits from spontaneous nonproteolytic activation (3) or the activated A subunits from deactivation (4). The well-known main function of factor XIII in blood consists in the stabilization of a formed thrombus by cross-linking of fibrin chains. Factor XIII also appears to be involved in cell adhesion and migration (5–7), assembly of extracellular matrix (8, 9), and tissue repair and wound healing (10, 11). The latter effects have been attributed to the ability of factor XIII to cross-link a variety of proteins of the extracellular matrix, e.g., fibronectin, collagen, and vitronectin (12–14).

During the last decade, several clinical observations showed that systemically applied factor XIII can reduce capillary hyperpermeability and may thus confer an antiedematous effect (for reviews, see references 15 and 16). It was found that the enhanced capillary permeability in patients with connective tissue disease is attenuated to almost normal levels under therapy with factor XIII (16, 17). It was also reported that factor XIII therapy reduces mucosal edema in inflammatory bowel disease (18, 19) and Henoch-Schönlein purpura (17, 20, 21). In an animal study, Hirahara et al. (22) have shown that factor XIII can suppress the enhanced vas-

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/99/05/1373/10 $2.00
Volume 189, Number 9, May 3, 1999 1373–1382
http://www.jem.org

cular permeability of guinea pig skin provoked by an inflammatory response upon injections of an antiendothelial cell antiserum. The underlying mechanism of these various antiedematous effects of factor XIII has remained unknown.

In this study, the question was addressed whether factor XIII can directly influence endothelial barrier function. Cultures of endothelial cells and the coronary system of an isolated heart were used as experimental models. In monolayers of cultured endothelial cells from porcine aorta, the paraendothelial passage of albumin was monitored as a parameter of endothelial barrier function (23, 24). Variations of macromolecule permeability in this model are attributable to changes in paracellular permeability (25). In the isolated rat heart, changes in tissue water content were determined as indication of vascular permeability (26). We found that the activated factor XIII reduces permeability of endothelial monolayers. Specifically, it prevents hyperpermeability provoked by energy depletion in endothelial monolayers and in ischemic-reperfused hearts.

Materials and Methods

Cell Cultures. Porcine aortic endothelial cells were isolated as described previously (27) by gentle mechanical scraping of the intima of the descending part of porcine aorta. Harvests of endothelial cells were plated at a density of 10^6 cells per 100-mm plastic dish. The cells were cultured at 37°C in a humidified atmosphere of 5% CO_2 in air. The "basal culture medium" consisted of medium 199 with Earle's salt, supplemented with 100 IU/ml penicillin G, 100 μg/ml streptomycin, and 20% (vol/vol) newborn calf serum (NCS).[1] The medium was renewed every other day. After 4 d, when the cells had grown to confluence, they were trypsinized in PBS (composed of [mM]: 137 NaCl, 2.7 KCl, 1.5 KH_2PO_4, and 8.0 Na_2HPO_4, at pH 7.4, supplemented with 0.05% [wt/vol] trypsin and 0.02% [wt/vol] EDTA). Endothelial cells were seeded at a density of 7×10^4 cells/cm^2 on either 24-mm round polycarbonate filters (pore size 0.4 μm) or 20-mm round glass coverslips for determination of albumin flux and immunostaining, respectively, and were cultured in basal culture medium (for compositions, see above). Experiments were performed with confluent monolayers, 4 d after seeding. The purity of these cultures was >99% endothelial cells as determined by uptake of DiI-ac-LDL, contrasted with <1% cells positive for α–smooth muscle actin.

Macromolecule Permeability of Endothelial Monolayers. The permeability of the endothelial cell monolayer was studied in a two-compartment system separated by a filter membrane (24, 28). Both compartments contained as basal medium modified Tyrode's solution (composition in mM: 150 NaCl, 2.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 1.0 $CaCl_2$, and 30.0 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; pH 7.4, 37°C) supplemented with 2% (vol/vol) NCS. There was no hydrostatic pressure gradient between the two compartments. The "luminal" compartment containing the monolayer had a volume of 2.5 ml, and the "abluminal" had a volume of 6.5 ml. The fluid in the abluminal compartment was constantly stirred. Trypan blue–labeled albumin (60 μM) was added to the luminal compartment. The appearance of the labeled albumin in the abluminal compartment was continuously monitored by pumping the liquid through a spectrophotometer (Specord 10; Carl Zeiss). Increases of the concentration of labeled albumin were detected with a time delay of <15 s. The concentration of labeled albumin in the luminal compartment was determined every 10 min of incubation. It did not change significantly in the time frame of the experiments.

The albumin flux (F, expressed as mol/[s × cm^2]) across the monolayer with the surface (S) was determined from the rise of albumin concentration ($d[A]_2$) during the time interval (dt) in the abluminal compartment (volume V):

$$F = (d[A]_2)/dt \times V)/S.$$

To facilitate the comparison of data obtained in this study with those of other studies, the permeability coefficient (P, expressed as cm/s) of the combined system of monolayer and filter support was calculated from F according to Fick's law of diffusion as follows:

$$P = F/([A]_1 - [A]_2)$$

where $[A]_1$ and $[A]_2$ denote tracer concentrations in the luminal and abluminal compartments, respectively. Because the driving force ($[A]_1 - [A]_2$) remained virtually unchanged in the course of the described experiments, the relative changes in F correspond to similar changes in the permeability coefficient.

Experimental Conditions. The basal medium used in incubations was modified Tyrode's solution (see above). Macromolecule permeability of the endothelial monolayer, transferred to the incubation chamber, was determined after an initial equilibration period of 20 min. The basal albumin permeability of each monolayer filter system was then determined for another 20 min of incubation. Agents were added as indicated, and the response of the albumin permeability was recorded for an additional 80 min.

In a set of experiments, endothelial monolayers were preincubated in basal medium (for composition, see above) supplemented with thrombin-activated factor XIII A (1 U/ml) at 37°C in a cell culture incubator for 2, 4, and 6 h. The endothelial monolayers were then transferred to the incubation chamber, and albumin permeability of these pretreated monolayers was determined after an initial equilibration period of 20 min.

Myocardial Water Content. Hearts from 250-g male Wistar rats were mounted immediately after isolation on a Langendorff perfusion system in a temperature-controlled chamber (37°C), as described previously (29). During normoxic perfusion, the chamber was flushed with humidified air, and during anoxic perfusion, with a 95% N_2 (vol/vol)/5% CO_2 (vol/vol) mixture. Under normoxic conditions, the hearts were perfused at a constant flow of 10 ml/min with an oxygenated saline medium (composition in mM: 140.0 NaCl, 24.0 $NaHCO_3$, 2.7 KCl, 0.4 KH_2PO_4, 1 $MgSO_4$, 1.8 $CaCl_2$, 5 glucose, pH 7.4; gassed with 95% O_2 [vol/vol]/5% CO_2 [vol/vol]). For low-flow ischemia, this normoxic period was followed by 40 min anoxic perfusion at 0.5 ml/min (composition of the perfusion medium as above; pH 7.4; gassed with 95% N_2 [vol/vol]/5% CO_2 [vol/vol]). After low-flow ischemia, hearts were again resupplied with oxygen by returning to the initial perfusion conditions. Factor XIII A was added to the perfusion medium 5 min before the onset of low-flow ischemia. It remained in the perfusion medium during the entire period of low-flow ischemia and reperfusion.

Activation of Factor XIII. Activation of the plasma factor XIII and factor XIII A was performed by incubations of known amounts of factor XIII in the presence of sepharose-coupled thrombin at 37°C in Tris buffer (200 mM, pH 7.4) for 20 min. The activated factor XIII was then separated from thrombin-sepharose by centrifugation. The contamination with thrombin of these supernatants was below detection limits. Factor XIII ac-

[1] *Abbreviations used in this paper:* DAB, 3,3′-diaminobenzidine; 2-DG, 2-deoxy-d-glucose; MI, metabolic inhibition; NCS, newborn calf serum.

tivity was determined by using the assay described by Fickenscher et al. (30) without thrombin in the assay.

Inactivation of Factor XIII A. Factor XIII A was inactivated using the alkylating agent iodoacetamide as described by Curtis et al. (31). To inactivate factor XIII, aliquots of the thrombin-activated factor XIII A containing ~12 μM (corresponding to 1 mg protein/ml) were incubated in the presence of 24 μM iodoacetamide at 37°C for 10 min. 48 μM glutathione was then added to react with the residual amounts of iodoacetamide, and incubations were continued for 5 min at room temperature. After this procedure, the activity of factor XIII A was below detection limits. Aliquots of the inactivated factor XIII A (~10 μg protein equivalent to 1 U factor XIII A) were added to the cells. The final concentrations of iodoacetamide and glutathione were 0.24 and 0.48 μM, respectively. At those concentrations, neither substance affected basal permeability of the endothelial monolayers.

Immunofluorescence Microscopy. Confluent endothelial monolayers were washed three times with PBS, then fixed with 5% paraformaldehyde for 10 min at 20°C, and washed again three times with PBS. The cells were covered with 100 μl polyclonal rabbit anti-factor XIII A or anti-factor XIII B antibodies (diluted 1:200 in PBS), and incubated for 6 h at 37°C. The coverslips were then washed three times with PBS, covered with 100 μl of mouse anti–rabbit IgG coupled to FITC (diluted 1:100 in PBS), and incubated for 6 h at 37°C. The coverslips were finally embedded in a 40% glycerol/PBS solution (pH 8.5) on glass slides. Cell monolayers were visualized using an inverse fluorescence microscope (model IX 70; Olympus).

Electron Microscopy. After permeability experiments, confluent endothelial monolayers on filter membranes were washed three times with PBS, and fixed with 5% paraformaldehyde for 10 min at 20°C as described for immunofluorescence microscopy. The cells were covered with 100 μl polyclonal rabbit anti-factor XIII A or anti-factor XIII B antibodies (diluted 1:200 in PBS), and incubated overnight at room temperature. The filters were then washed three times with PBS, covered by 100 μl of donkey anti–rabbit IgG coupled to peroxidase (diluted 1:150 in PBS), and incubated at room temperature for 1 h. The filters were washed twice with PBS and twice with Tris-HCl (10 mM, pH 7.4) and then incubated with 3,3′-diaminobenzidine (DAB) and hydrogen peroxide as substrates for the peroxidase reaction in the presence of nickel ammonium sulfite for 45 min. The filters were then washed again three times with Tris-HCl and exposed to a 1% solution of OsO_4 at 4°C for 1 h. After washing twice with Tris-HCl and twice with maleate buffer (pH 5.2), the specimens were incubated in a 1% uranyl acetate solution in maleate buffer in the dark at room temperature for 1 h. Subsequently, the specimens were washed again three times with maleate buffer, dehydrated in 70% ethanol, and transferred to 2,2′-dimethoxypropan, followed by embedding in spurr resin. Polymerization of the embedded specimens was performed at 60–70°C overnight. Ultrathin cross-sections of the monolayers were cut, stained with lead citrate, and viewed with a transmission electron microscope (model EM 902; Carl Zeiss).

Statistical Analysis. Data are given as means ± SD of $n = 6$ experiments using independent cell preparations. Statistical analysis of data was performed according to Student's unpaired t test. Probability (P) values <0.05 were considered significant.

Materials. Donkey anti–rabbit IgG coupled to peroxidase was from Amersham Buchler; Falcon plastic tissue culture dishes were from Becton Dickinson; polyclonal anti-factor XIII A antibody, and polyclonal anti-factor XIII B antibody DADE were from Behring Diagnostics; glutathione was from Boehringer Mannheim; plasma factor XIII and isolated factor XIII B subunit purified from Fibrogammin HS™, factor XIII A subunit (recombinant human factor XIII expressed in yeast and purified to homogeneity [impurities <100 ppm]), and human thrombin were from Centeon Pharma GmbH; Transwell® polycarbonate filter inserts (24-mm diameter, 0.4-μm pore size) were from Costar; NCS), medium 199, penicillin-streptomycin, and trypsin-EDTA were from GIBCO Life Technologies; DAB (ISOPAC™) and DiI-ac-LDL (acetylated low-density lipoprotein labeled with 1,1′-dioctadecyl-1-3,3,3′,3′-tetramethyl-indocarbocyanine perchlorate) were from Paesel & Lorei; spurr resin was from Serva; anti–rabbit IgG coupled to peroxidase or FITC, and iodoacetamide were from Sigma. All other chemicals were of the best available quality, usually analytical grade.

Results

Effect of Factor XIII on Monolayer Permeability. It was tested initially whether the activity of factor XIII added to endothelial monolayers is changed throughout the time course of a permeability experiment. The following additions to the luminal compartment of the incubation chambers were made: thrombin-activated or nonactivated plasma factor XIII, and thrombin-activated or nonactivated factor XIII A subunit. As shown in Fig. 1, the measured activities remained stable during the entire experimental period.

Macromolecule permeability of endothelial monolayers was continuously monitored by determining the flux of albumin across the monolayers. Under control conditions, mean permeability was $5.9 \pm 0.6 \times 10^{-6}$ cm/s (Fig. 2). It remained constant during the entire period of observation. Addition of the thrombin-activated plasma factor XIII (1 U/ml) caused a rapid decrease of albumin permeability, which was reduced by 30% after 20 min. In contrast to the activated plasma

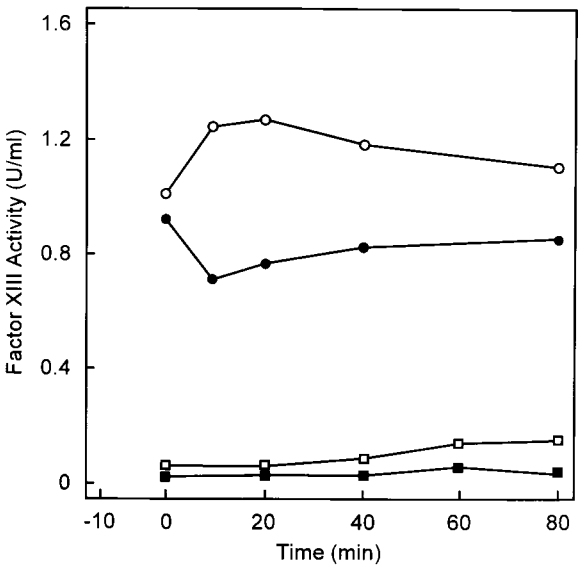


Figure 1. Factor XIII activity in the luminal compartment of the experimental two-compartment system. Thrombin-activated plasma factor XIII (●, 0.8 U/ml), thrombin-activated factor XIII A subunit (○, 1.2 U/ml), nonactivated plasma factor XIII (■, 10 μg/ml), or nonactivated factor XIII A subunit (□, 10 μg/ml) was added to the luminal compartment containing the endothelial monolayer at time point 0.

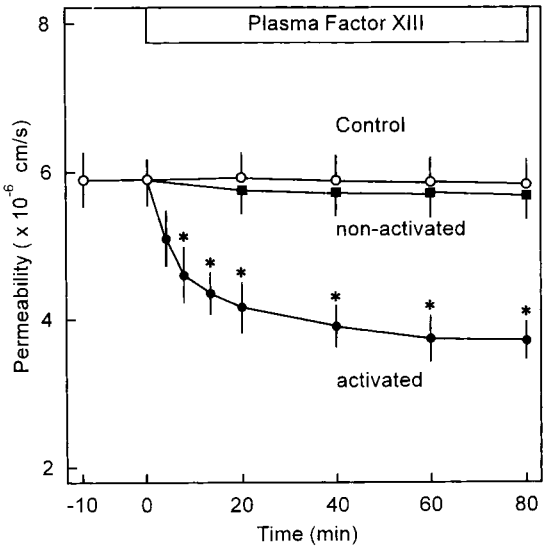


Figure 2. Effect of the plasma factor XIII on albumin permeability of porcine aortic endothelial monolayers. At time 0, the following additions were made: none (○, Control); nonactivated plasma factor XIII (■, 20 μg protein/ml = 1 U/ml); activated factor XIII (●, 1 U/ml). Data are means ± SD of $n = 5$ separate experiments of independent cell preparations. *$P < 0.05$ vs. control.

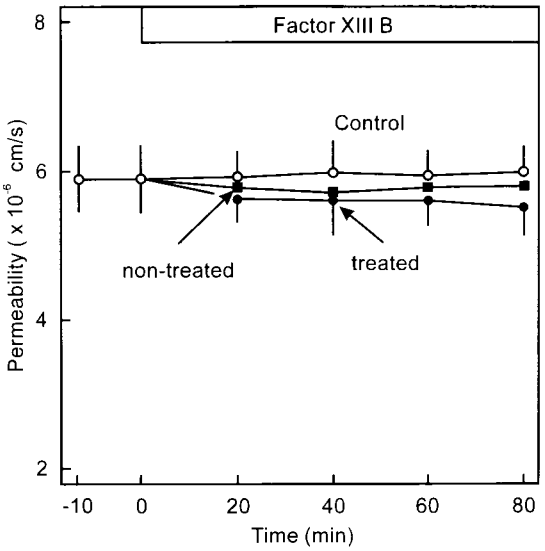


Figure 4. Effect of the factor XIII B subunit on albumin permeability of endothelial monolayers. At time 0, the following additions were made: none (○, Control); factor XIII B subunit (■, 10 μg protein/ml) not treated with thrombin; factor XIII B subunit (●, 10 μg protein/ml) treated with thrombin. Data are means ± SD of $n = 5$ separate experiments of independent cell preparations. *$P < 0.05$ vs. control.

factor XIII, addition of the nonactivated plasma factor XIII had no effect on permeability.

Exposure of endothelial monolayers to the thrombin-activated factor XIII A subunit (10 μg/ml, equivalent to ~1 U/ml) also led to a rapid reduction of permeability, by 34% within 20 min (Fig. 3). The nonactivated factor XIII A (10 μg/ml) as well as additions of the iodoacetamide-inactivated factor XIII A (10 μg/ml) had no effect on permeability. Likewise, the isolated factor XIII B subunit (10 μg/ml) did not affect the albumin permeability of the endothelial monolayers (Fig. 4). In a set of experiments, it was tested whether the activated factor XIII A can affect albumin permeability of endothelial monolayers for a prolonged period of time. For that reason, endothelial monolayers were preincubated with thrombin-activated factor XIII A for 2, 4, and 6 h. Albumin permeability was then determined. As shown in Table I, the reduction of albumin permeability induced by the activated factor XIII A persists for 6 h.

The activated factor XIII A reduced albumin permeability with increasing activity (Fig. 5), with half-maximal effect at 0.9 U/ml. In contrast, the nonactivated factor XIII A as well as factor XIII B had no significant effect on albu-

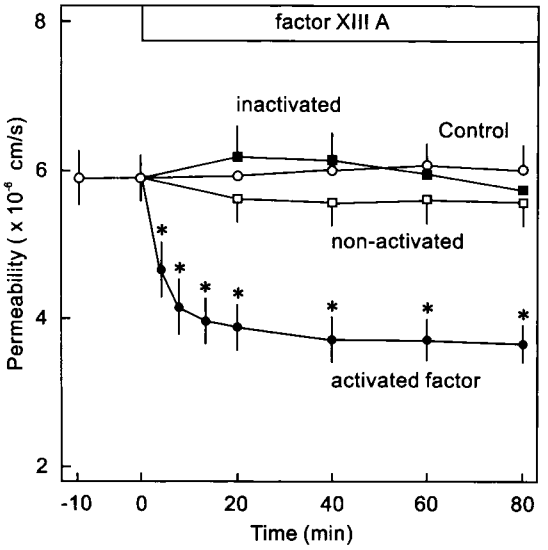


Figure 3. Effect of the factor XIII A subunit on albumin permeability of endothelial monolayers. At time 0, the following additions were made: none (○, Control); nonactivated factor XIII A (□, 10 μg/ml = 1 U/ml); iodoacetamide-inactivated factor XIII A (■, 10 μg/ml = 1 U/ml); thrombin-activated factor XIII A (●, 10 μg/ml = 1 U/ml). Data are means ± SD of $n = 5$ separate experiments of independent cell preparations. *$P < 0.05$ vs. control.

Table I. *Effect of Activated Factor XIII A on Albumin Permeability of Endothelial Monolayers after Various Times of Incubation*

Time	Permeability
h	*×10^{-6} cm/s*
0	5.9 ± 0.4
2	3.7 ± 0.5*
4	3.5 ± 0.6*
6	3.1 ± 0.3*

Endothelial monolayers were preincubated in the presence of thrombin-activated factor XIII A (1 U/ml) for 2, 4, and 6 h. Albumin permeability was then determined. Data are means ± SD of $n = 5$ separate experiments of independent cell preparations. *$P < 0.05$ vs. time 0.

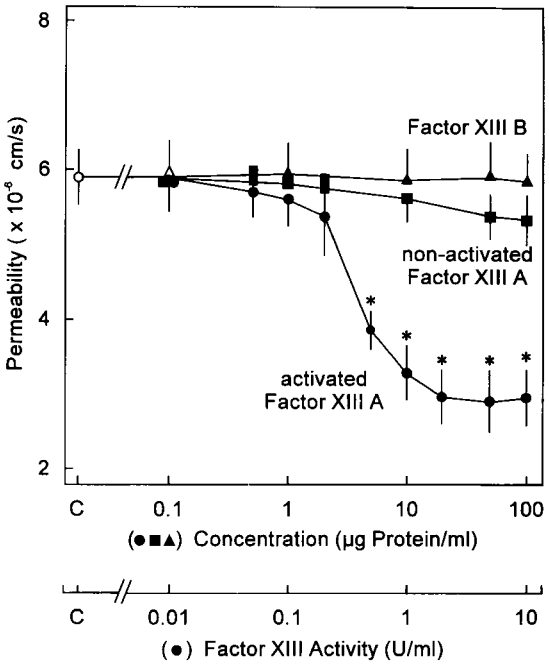


Figure 5. Dose-dependent effect of factor XIII on albumin permeability. Permeability was determined 20 min after the following additions: none (○, control [C]); nonactivated factor XIII A subunit (■); thrombin-activated factor XIII A subunit (●, 10 µg protein/ml = 1 U/ml); factor XIII B subunit (▲). Data are means ± SD of $n = 5$ separate experiments of independent cell preparations. *$P < 0.05$ vs. control.

min permeability when applied in the same range of protein concentration.

Immunostaining of Endothelial Monolayers. For immunostaining, a polyclonal rabbit anti-factor XIII A antibody was used which recognizes the activated as well as the nonactivated factor XIII A (32). Immunostaining of endothelial monolayers incubated for 20 min in the presence of thrombin-activated factor XIII A (1 U/ml) revealed factor XIII A–positive staining along the interface of adjacent endothelial cells (Fig. 6 A). In monolayers that were exposed to nonactivated factor XIII A at equivalent protein concentration (10 µg protein/ml), immunostaining for factor XIII A remained absent (Fig. 6 B). As control, endothelial monolayers that had not been incubated in the presence of factor XIII A were exposed to either the first anti-factor XIII A and second antibody (FITC-coupled anti–rabbit IgG; Fig. 6 C) or the second antibody alone (Fig. 6 D). No specific staining was observed with these protocols.

In a second set of experiments, endothelial monolayers were incubated in the presence of factor XIII B (10 µg protein/ml) which had been preexposed or not to thrombin. For immunohistochemistry, a specific polyclonal antibody raised against factor XIII B (32) was used, which we confirmed to stain isolated factor XIII B (not shown). No specific staining for factor XIII B was detected in the monolayers (Fig. 7).

To analyze the localization of factor XIII A in cross-sections of endothelial monolayers in greater detail, these were incubated for 20 min in the presence or absence of thrombin-activated or nonactivated factor XIII A. The endothelial monolayers were then processed for transmission electron microscopy. When activated factor XIII A had been applied, factor XIII A immunoreactivity was identified by the accumulation of an electron-dense DAB reaction product at the intercellular cleft and of the basal endothelial surface along the margin of the cells (Fig. 8 C). In contrast, no DAB reaction product was observed in intercellular clefts of control monolayers (Fig. 8 A) or in endothelial monolayers exposed to nonactivated factor XIII A (Fig. 8 B).

Effect of Factor XIII A on Hyperpermeability Induced by Endothelial Energy Depletion. As shown in previous studies from our laboratory (28, 33), metabolic inhibition (MI) of mitochondrial and glycolytic energy production causes a rapid rise in macromolecule permeability. In the present study, it was tested whether the activated factor XIII A can attenuate the hyperpermeability in energy-depleted endothelial monolayers. Addition of 1 mM KCN (inhibitor of mitochondrial respiration) plus 1 mM 2-deoxy-d-glucose (2-DG, inhibitor of glycolytic ATP production) caused an increase in permeability by 23% within 10 min (Fig. 9). Exposure of endothelial monolayers to 1 U/ml of activated factor XIII A led to a 30% reduction of permeability. In the presence of activated factor XIII A, addition of the metabolic inhibitors no longer caused an increase in permeability. The level of permeability remained even as low as that obtained by addition of the activated factor XIII A before MI.

In immunomicroscopy, the staining of factor XIII A at cell–cell interfaces was enhanced when the monolayers were exposed to metabolic inhibitors (Fig. 10). As can be seen by comparison of immunostaining and phase–contrast images of the same section, the enlarged zones of factor XIII A–positive staining correspond to gaps opening between adjacent cells.

Effect of Factor XIII A on Myocardial Water Content. To analyze whether the activated factor XIII A can also affect endothelial barrier function in the coronary system, the isolated perfused heart was used and changes of myocardial water content were determined. Under control conditions, the myocardial water content of the normoxic perfused rat heart was, on average, 430 ml/100 g dry wt over a period of 160 min of observation (Fig. 11). To provoke an increase in vascular permeability, hearts were exposed to a 40-min period of low-flow ischemia followed by a period of 60 min of normoxic reperfusion. Ischemia-reperfusion experiments were performed with addition of either the nonactivated or the activated factor XIII A 5 min before onset of anoxic low-flow perfusion. With the nonactivated factor XIII A, the water content of reperfused hearts rose to 530 ml/100 g dry wt. In the presence of the activated factor XIII A (5 U/ml), myocardial water content remained as it was before reperfusion.

Discussion

The central question of this study was whether factor XIII can directly influence endothelial barrier function. In

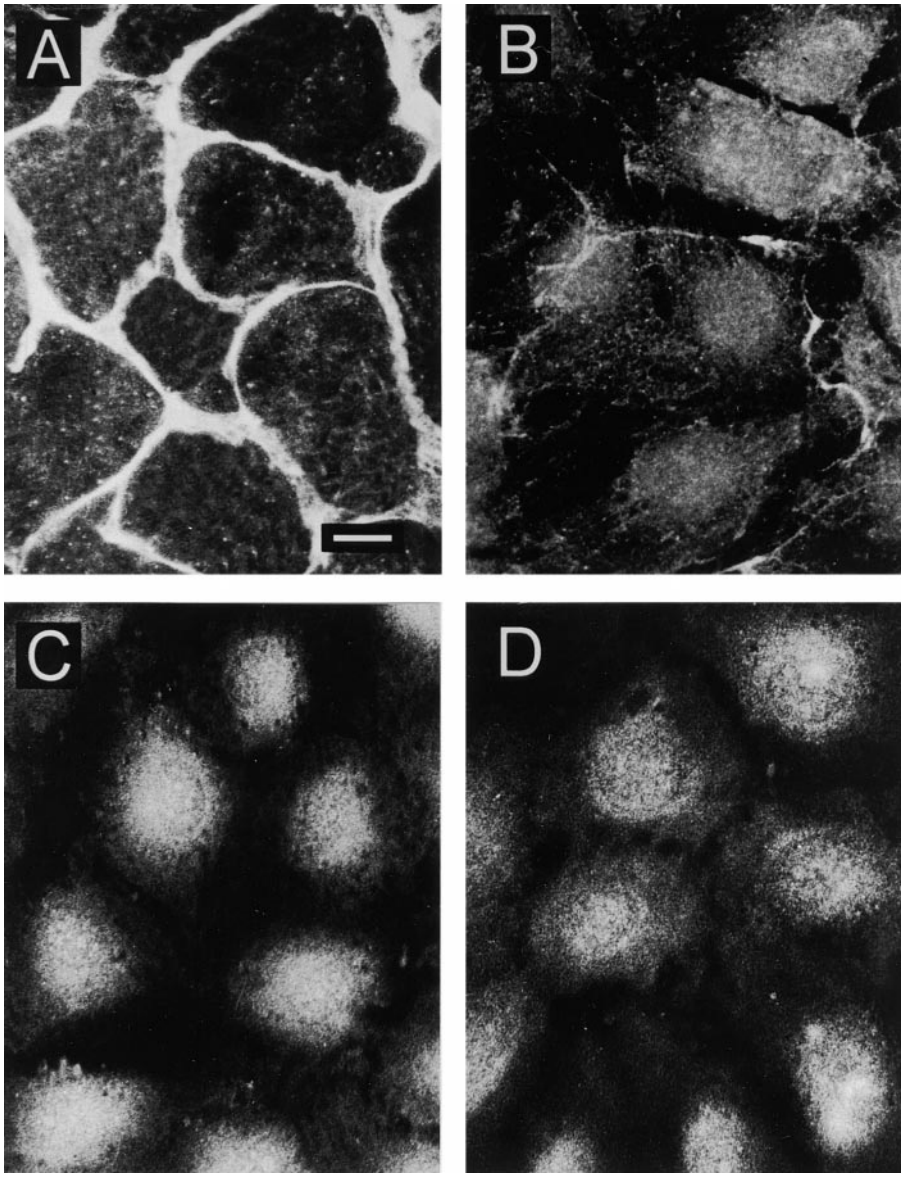


Figure 6. Immunostaining of factor XIII A in endothelial monolayers. (A) Endothelial cells were incubated for 20 min in the presence of activated factor XIII A (1 U/ml). Factor XIII A–positive staining is seen along the interfaces of adjacent endothelial cells. (B) Endothelial cells were exposed to nonactivated factor XIII A (10 μg/ml). No positive staining for factor XIII A is observed. (C) Endothelial cells not preincubated with factor XIII A were exposed to anti-factor XIII A and FITC-coupled anti–rabbit IgG antibody (first and second antibody control). Only background fluorescence is seen. (D) Endothelial cells not preincubated with factor XIII A were exposed only to the FITC-coupled anti–rabbit IgG antibody (second antibody control). Again, only background fluorescence is apparent. Bar, 10 μm.

the model of cultured endothelial monolayers, we found that activated factor XIII not only lowers the basal permeability for macromolecules but also prevents the increase in permeability provoked by an inhibition of endothelial energy production. In the isolated whole heart, activated factor XIII was able to prevent edema formation caused by ischemia-reperfusion. The endothelial effects of factor XIII are exerted only by the activated form of the A subunit.

Confluent monolayers of cultured porcine aortic cells were used as a model (24, 28, 33). To characterize the barrier of these monolayers towards macromolecules, the passage of albumin across the monolayers was studied. Changes in macromolecule permeability in this model are attributed to changes in paracellular permeability (25). The basal level of permeability in this model is not the lowest possible, and can therefore be used to investigate factors improving endothelial barrier function without prior stimulation (23, 34).

The nonactivated plasma factor XIII did not affect permeability of the monolayers. However, when activated by exposure to sepharose-coupled thrombin, plasma factor XIII markedly lowered the permeability. To analyze which part of the heterodimeric complex is responsible for this effect, a recombinant A subunit and a purified B subunit of factor XIII were applied in the permeability experiments. The A subunit was equipotent to plasma factor XIII when activated by exposure to thrombin. The lowering effect on permeability of the factor XIII A was dependent on its enzymatic activity. If factor XIII A was inactivated by the alkylating agent iodoacetamide, it no longer reduced permeability. The B subunit had no effect. The results thus show that the activated A subunit of factor XIII represents the active principle of the permeability-lowering effect.

Active factor XIII is a transglutaminase capable of cross-linking various types of proteins (2) and is entrapped in the stable protein meshwork formed. With immunomicroscopy, we found factor XIII deposited at the endothelial

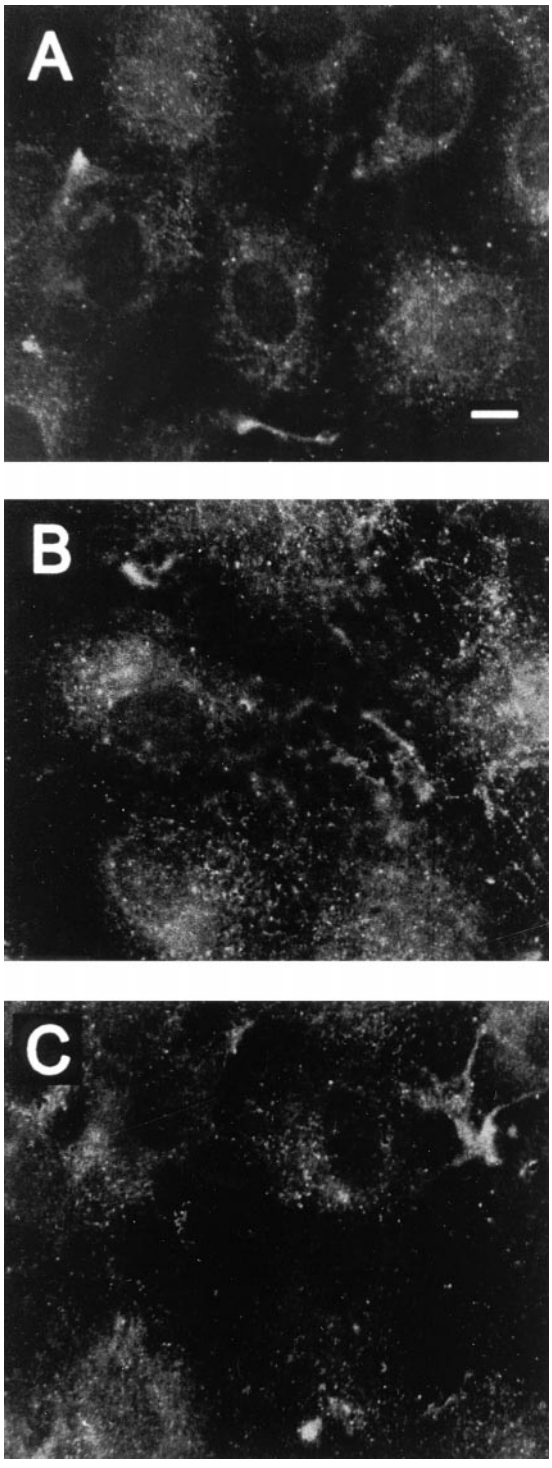


Figure 7. Immunostaining of factor XIII B in endothelial monolayers. (A) Endothelial cells were incubated for 20 min in the presence of isolated factor XIII B subunit (10 μg/ml) which had been pretreated with thrombin. Only background fluorescence is observed. (B) Endothelial cells were exposed to factor XIII B subunit (10 μg/ml) which was not pretreated. Only background fluorescence is present. (C) Endothelial cells not preincubated with factor XIII B subunit were exposed to anti-factor XIII B and FITC-coupled anti–rabbit IgG antibody (first and second antibody control). There is only background fluorescence. Bar, 50 μM.

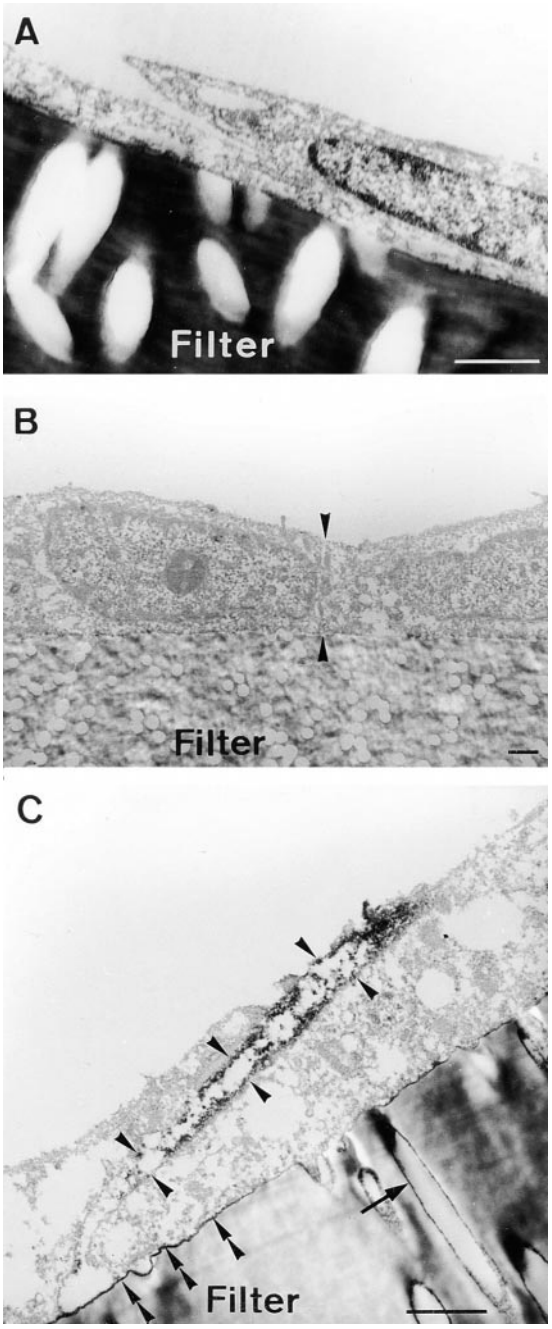


Figure 8. Electron microscopic localization of factor XIII A immunoreactivity in cross-sections of endothelial monolayers. Cross-sections of the interface of two adjacent endothelial cells are shown. (A) Control conditions in the absence of factor XIII A. (B) After incubation for 20 min in the presence of nonactivated factor XIII A (10 μg/ml) or (C) of activated factor XIII A (1 U/ml). Factor XIII A immunoreactivity was identified by accumulation of electron-dense DAB reaction product in the intercellular clefts (arrowheads) only in those monolayers exposed to the activated factor XIII A (C). Reaction product in C is also found at the basal endothelial surface between the cells and the filter (double arrowheads), and the inner surface of the filter pores (arrow). Bars, 1 μm.

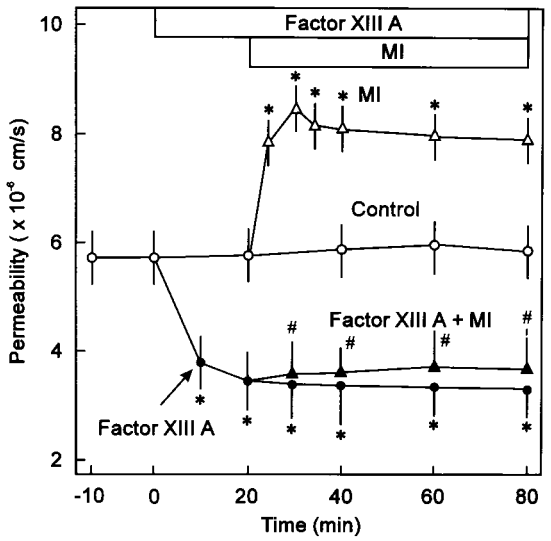


Figure 9. Effect of activated factor XIII A (1 U/ml) on monolayer permeability of endothelial cells under MI (1 mM KCN plus 1 mM 2-DG). Conditions were as follows: no MI and no factor XIII A (○, Control); MI and no factor XIII A (△); addition of factor XIII A and no MI (●); addition of factor XIII A and MI (▲). Data are means ± SD of $n = 5$ separate experiments of independent cell preparations. $^{*}P < 0.05$ vs. control; $^{\#}P > 0.05$, not significant vs. factor XIII A.

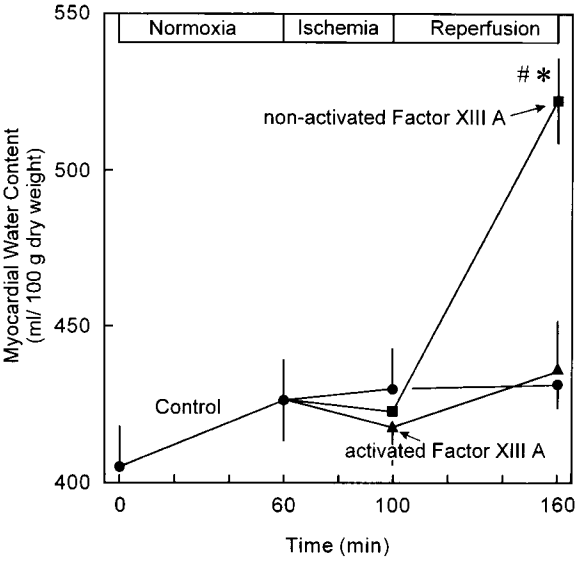
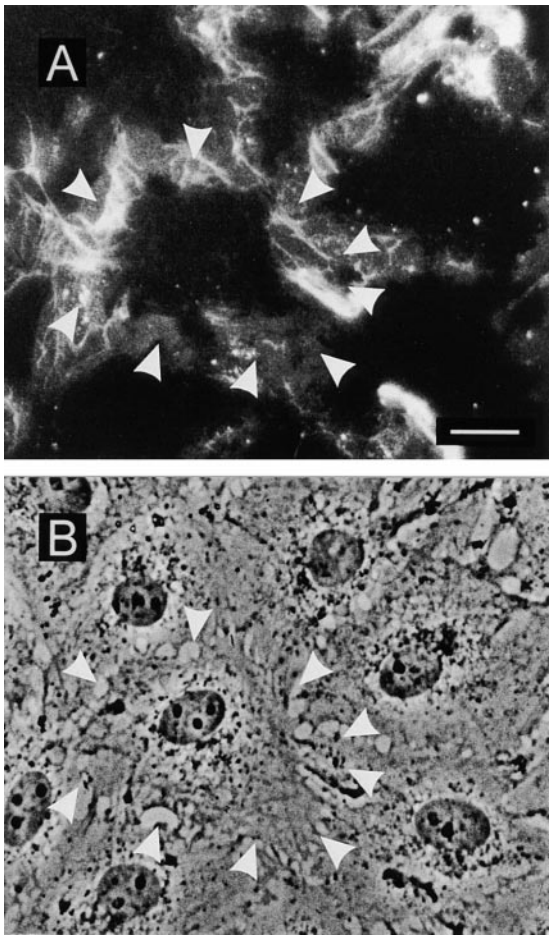


Figure 11. Effect of factor XIII on changes of myocardial water content under low-flow ischemia and reperfusion. Rat hearts in Langendorff mode were perfused for 60 min with normoxic perfusate at 10 ml/min, followed by 40 min low-flow perfusion at 0.5 ml/min, and 60 min reperfusion with normoxic perfusate at 10 ml/min. Control hearts were perfused up to 160 min with normoxic media at 10 ml/min (●). Perfusate of low-flow ischemia and reperfusion was supplemented with either nonactivated factor XIII A (■, 50 μg/ml) or thrombin-activated factor XIII A (▲, 50 μg/ml = to 5 U/ml). Data are means ± SD of $n = 4$ separate experiments of independent heart preparations. $^{*}P < 0.05$ vs. 160 min normoxia; $^{\#}P < 0.05$ vs. activated factor XIII A.



monolayer under exactly those conditions where factor XIII reduced monolayer permeability, i.e., when the activated A subunit was present. Immunoreactivity of factor XIII A was localized under these circumstances along the interfaces of adjacent endothelial cells. Electron microscopy revealed that it was concentrated in the narrow gaps between adjacent cells and at the basal endothelial surface between the cells and the filter support. Mass deposition of factor XIII A was not found at any other site within the endothelial monolayers. The B subunit did not form depositions on the monolayer when applied. There are a variety of proteins like fibronectin and vitronectin residing in the intercellular clefts and the subendothelial matrix which are involved in cell-to-cell and cell-to-matrix adhesion of endothelial cells and which represent substrates for factor XIII cross-linking reactions (12, 14). Interestingly, the small intercellular clefts represent the principle paracellular pathway for passage of macromolecules in these monolayers. Therefore, the microscopic observations suggest that active factor XIII A reduces monolayer permeability because it reacts with extracellular matrix proteins at these strategic sites

Figure 10. Immunostaining of factor XIII A in endothelial monolayers under MI. (A) Endothelial cells were first incubated in the presence of activated factor XIII A (1 U/ml), then the monolayer was exposed to MI for 60 min (see Fig. 9). Broad bands of factor XIII A–positive staining are seen at cell–cell interfaces. (B) Phase–contrast image corresponding to A. Enlarged zones of factor XIII A–positive staining (arrowheads) in A correspond to gaps (arrowheads) in B between adjacent cells. Bar, 10 μm.

of the endothelial barrier. In doing so it may itself become entrapped, as in fibrin clots.

We showed previously, using the same experimental model, that energy depletion of endothelial cells causes a rapid rise in monolayer permeability (28, 33). This rise in permeability is associated with a widening of intercellular gaps. We find now that in the presence of active factor XIII A, the rise in permeability is abolished even though the energy-depleted cells in the monolayer remain retracted from each other. The latter observation indicates that factor XIII does not prevent the immediate structural consequences of energy loss within endothelial monolayers. The explanation for the protective effect of factor XIII seems to lie in another finding, that the intercellular gaps contain massive depositions of factor XIII immunoreactivity. This finding is consistent with the above hypothesis that factor XIII reduces monolayer permeability by cross-linking of proteins at the paracellular passageways.

To study whether the activated factor XIII A can affect endothelial barrier function in an intact coronary system, saline-perfused rat hearts were used as a model. Low-flow ischemia and subsequent reperfusion caused a marked increase in myocardial water content, as also reported by others (26). When the perfusion medium was supplemented with the activated factor XIII A before onset of low-flow ischemia, an increase in myocardial water content did not occur. These data show that the activated factor XIII A can prevent development of hyperpermeability in this perfused heart model.

This study has revealed a new function of factor XIII, i.e., stabilization of endothelial barrier function. It shows that this function is due to a direct effect on the endothelial monolayer. The observations in microscopy indicate that factor XIII can reduce the permeability through an endothelial monolayer by interactions with proteins of the extracellular matrix between cells. As the permeability-lowering effect is restricted to the active form of factor XIII, which acts as an enzymatic cross-linker of proteins, this effect seems to be due to narrowing of the sieving meshwork in the paracellular transendothelial passageways. The experiments on energy-depleted monolayers and ischemic-reperfused hearts indicate that the active factor XIII can be used to prevent edema formation caused by endothelial metabolic disturbances.

This work was supported by the Deutsche Forschungsgemeinschaft, grants A3 and A4 of SFB 547.

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Received for publication 22 June 1998 and in revised form 3 March 1999.

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