

Eur. J. Clin. Chem. Clin. Biochem.
Vol. 32, 1994, pp. 495–500

© 1994 Walter de Gruyter & Co.
Berlin · New York

Tissue Kallikrein Activity and Kinin Release in Human Endothelial Cells

By K. Graf¹, M. Gräfe¹, W. Auch-Schwelk¹, C. R. Baumgarten², H. Scheffer², A. Hildebrandt³ and E. Fleck¹

¹ Department of Internal Medicine and Cardiology, German Heart Institute, Berlin, Germany

² Department of Clinical Immunology, Universitätsklinikum Rudolf Virchow, Freie Universität Berlin, Berlin, Germany

³ Bundesgesundheitsamt, Berlin, Germany

(Received January 18/April 13, 1994)

Summary: The kininogenase, tissue kallikrein (EC 3.4.21.8), has been identified in different blood vessels. The enzyme was mainly found in vascular smooth muscle cells. It is not known whether it is present and functionally active in vascular endothelial cells. The following study investigates the presence of tissue kallikrein in endothelial cells from human umbilical veins and pulmonary arteries. Tissue kallikrein was demonstrated in three ways:

- 1) by immunostaining in endothelial cells;
- 2) by measurement of tissue kallikrein activity using a colorimetric assay;
- 3) by the measurement of kinin release in intact and homogenised endothelial cells with a radioimmunoassay.

Immunostaining demonstrated the presence of tissue kallikrein in endothelial cells from human umbilical veins and endothelial cells from human pulmonary arteries. Tissue kallikrein-like activity, measured by the degradation of *D*-Val-cyclohexyl-Ala-Arg-4-nitraniline, was 3.57 ± 0.5 mU/10⁶ endothelial cells from human umbilical veins and 7.52 ± 0.84 mU/10⁶ endothelial cells from human pulmonary arteries. Intracellular kinin concentrations were 424 ± 83 pg/10⁶ cells in endothelial cells from human umbilical veins and 576 ± 146 pg/10⁶ cells in endothelial cells from human pulmonary arteries, and they increased in a time-dependent manner after homogenisation. The increase was abolished by aprotinin (1000 kIU), an inhibitor of tissue kallikrein in both cell types. Addition of exogenous kallikrein (5 mU) to homogenised cells led to a five fold increase of kinin concentrations after five minutes, indicating a sufficient resource of cellular kininogen. Removal of extracellularly bound kininogen by washing with dextran sulphate (100 mg/l) resulted in an approximately 75% reduction of the cellular kinin release. This suggests that extracellular kininogen is the main source of endothelial kinin production. The present data demonstrate the presence of tissue kallikrein in human endothelial cells and provide evidence for the ability of human endothelial cells to release kinins.

Introduction

Kinins are potent vasodilators, which contribute to the local regulation of vascular tone (1, 2). Their vasorelaxant effect is mediated by the release of endothelium-derived relaxing factor and prostacyclin (3, 4). Kinins are released from kininogens by tissue (glandular) and plasma kallikreins, two serine proteases of restricted substrate specificity (5). Plasma kallikrein bound to high molecular weight kininogen circulates in the human

blood. Presence of tissue kallikrein was shown in different tissues and body fluids, e. g. pancreas, submandibular glands and urine (5). Although the presence of tissue kallikrein in the endothelium has not been directly demonstrated, several pharmacological studies using the bradykinin-receptor antagonist, HOE 140, and angiotensin converting enzyme inhibitors suggest the presence of a kinin generating system in the endothelium (6–8).

In the vasculature, the occurrence of tissue kallikrein-like enzyme has been described in the adventitia of the

rat (9) and in vascular smooth muscle cells isolated from the rat aorta (10). Saed (11) has demonstrated the presence of tissue kallikrein mRNA in rat arteries and veins and in isolated smooth muscle cells of the rat. Kallikrein-like activity is present in human tissue from aorta and renal arteries (12). Moshi and co-workers described a kininogenase in canine myocardium and aorta, which releases bradykinin and shows similarities with tissue kallikrein (13). However, most investigators attribute tissue kallikrein activity to the vascular smooth muscle cells, but not to endothelial cells, although the vascular endothelium seems to be one of the main targets of kinin action (1–4). Therefore we investigated the presence of tissue kallikrein in cultured human endothelial cells by three different methods: immunostaining, measurement of enzyme activity, and measurement of kinin release.

Materials and Methods

Cell culture

Human umbilical vein endothelial cells were prepared according to the method of Jaffe (14) as previously described (15). For the preparation of human pulmonary artery endothelial cells, segments of human pulmonary arteries (1.5–3.5 cm²) were incubated in 2 g/l collagenase (Roth, Karlsruhe, Germany) for 30 minutes at 37 °C. The segments were then rinsed with Medium 199 containing fetal calf serum, volume fraction 0.2 (Gibco-BRL, Berlin, Germany), and the detached cells were collected. The cell suspension was pelleted and seeded into a 35 mm dish (Falcon, Heidelberg, Germany) coated with 2 g/l gelatine (Sigma, Deisenhofen, Germany) and incubated in culture medium at 37 °C and 5% CO₂. The culture conditions for endothelial cells from human umbilical veins and endothelial cells from human pulmonary arteries were similar. The culture medium contained Medium 199, fetal calf serum (volume fraction 0.2), 10⁵ U/l penicillin, 100 mg/l streptomycin, 10 mmol/l HEPES (all Sigma), 0.2 mol/l L-glutamine (Seromed, Berlin, Germany), and 10 µg/l endothelial cell growth factor (Boehringer, Mannheim, Germany). All experiments were performed with cells of the second passage. The purity and identity of the endothelial cultures was determined by measuring factor VIII related antigen, using a specific antibody (Dako, Hamburg, Germany), and by measuring the uptake of fluorescence conjugated-low density lipoprotein in confluent monolayers (Paesel & Lorei, Mainz, Germany) (16). Endothelial cells were counted using a haemocytometer.

Measurement of tissue kallikrein activity in endothelial cells

Confluent monolayers of endothelial cells from human umbilical veins and endothelial cells from human pulmonary arteries in 35 mm dishes were washed twice with phosphate-buffered saline (Gibco-BRL), scraped off the dish with a rubber policeman, suspended in 0.5 ml Tris buffer (0.1 mol/l, pH 7.4), containing 1 ml/l Tween 20 (Serva, Heidelberg, Germany), then sonicated for 5 seconds (20% output energy) with a Sonifier B-12 (Branson Sonic Power Co., Danbury, Co., USA) on ice. Samples of the cell homogenate were assayed immediately for tissue kallikrein activity.

Kinin release in endothelial cell supernatants

All experiments were done in 1.5 ml serum-free incubation medium to avoid interference of the serum with the bradykinin RIA. The serum-free medium contained RPMI 1640 (Gibco-BRL), 10 mmol/l HEPES (Sigma), antibiotics and the peptidase inhibitors,

phosphoramidon (10 nmol/l) and lisinopril (1 µmol/l, ICI, Germany). Confluent monolayers were washed twice with phosphate-buffered saline, incubation medium was added, and the cells were incubated at 37 °C. After 10 minutes, 1 hour and 4 hours, aliquots of 0.2 ml were taken, frozen in liquid nitrogen, and stored at –70 °C until measurement of kinin concentrations by RIA.

Kinin release in endothelial cell homogenates

All experiments were done in 2 ml incubation medium. Confluent monolayers were washed twice with phosphate-buffered saline. Thereafter, the incubation medium was added and the cultures were allowed to equilibrate for 15 minutes. The cells were then scraped off the culture dish and homogenised by sonication as described above. Aliquots of 0.2 ml were taken immediately after homogenisation and after 5 and 30 minutes. Aliquots were mixed with 0.2 ml Tris-buffer (0.2 mol/l Tris, 0.01 mol/l EDTA, pH 7.4, Serva AG, Heidelberg, Germany) on ice, frozen in liquid nitrogen, and stored at –70 °C. Parallel experiments were performed in the presence of 1000 kIU/dish aprotinin (Bayer AG, Leverkusen, Germany), which was added 15 minutes prior to homogenisation. In a second set of experiments, confluent cell monolayers were washed twice with dextran sulphate (100 mg/l, Sigma) for 4 minutes at room temperature. Then the cells were incubated with the incubation medium and bradykinin release was measured as described above. To some cell homogenates, 0.5 or 5 U/l porcine tissue kallikrein (Sigma) was added, and aliquots for kinin determination were taken at 5, 30 and 60 minutes afterwards.

Immunostaining

Endothelial cells were placed in phosphate-buffered saline containing 10 g/l bovine serum albumin (Sigma). Cyto centrifugal specimens containing 2 × 10⁵ endothelial cells per slide were prepared using a Cytospin 2 (Shandon Southern Prod. Ltd., Runcorn, Cheshire, UK) at 150 g for 5 minutes. All cyto centrifuge slides were stained by the alkaline phosphatase anti-alkaline phosphatase method described by Cordell (17). The fixed cells were stained with an anti-human tissue kallikrein antiserum (dilution 1 : 1000) (18) or an antibody against factor VIII related antigen (dilution 1 : 100, Dako), which was used as positive control. Incubation with a preimmune antiserum (instead of kallikrein antibody) was performed as a negative control. After immunostaining, slides were counterstained with Mayer's acid haematoxylin. The number of positive cells was estimated by evaluation of 5 microscopic powerfields of each specimen (magnification × 200; endothelial cells from human umbilical veins: n = 5, endothelial cells from human pulmonary arteries: n = 3).

Assay for tissue kallikrein activity

Tissue kallikrein activity was assessed by the colorimetric measurement of the release of 4-nitroaniline (19). Twenty microlitres of standard (0.1, 0.25, 0.5, 1, 2, 4, 8 mU porcine tissue kallikrein, Sigma) or sample were diluted in a total volume of 150 µl (0.1 mol/l Tris, 0.234 mol/l NaCl, pH 7.8). Assays were performed using 96-well titre plates (Falcon, Heidelberg, Germany). The enzymatic reaction was started by addition of 150 µl of 0.5 mmol/l D-Val-cyclohexyl-Ala-Arg-4-nitraniline (Chromozym GK, Boehringer, Germany) in the presence and absence of 200 kIU aprotinin (Bayer AG). The absorbance at 405 nm (Titertek MCC 340; Lausanne, Switzerland) was measured to determine the 4-nitroaniline release after 1, 2, 3, and 6 hours. Tissue kallikrein activity was calculated from the maximal slope of 4-nitroaniline generation. One mU porcine tissue kallikrein (Sigma) induced an increase of $\Delta A_{405\text{ nm}}/10\text{ min} = 0.052 \pm 0.005$ (n = 12). Only the activity of the reaction inhibited by aprotinin was attributed to tissue kallikrein-like activity.

Kinin radioimmunoassay

Immunoreactive kinins were measured by a radioimmunoassay (RIA) described previously (15, 20). The detection limit of the RIA was 20 ng per litre cell supernatant medium. The inter- and intrassay coefficients of variation were 4% and 5%, respectively. The antiserum does not distinguish between lysyl-bradykinin (kallidin), bradykinin and methionyl-lysyl-bradykinin. The cross reactivity was less than 1% to des-9arg-bradykinin. The peptidase inhibitors added to the incubation medium did not interfere with the RIA at the concentrations used in the experiments. No cross reactivity of the bradykinin-antiserum to porcine tissue kallikrein was observed.

Calculations and statistics

Each experiment was done with duplicate or triplicate dishes. Results are shown as means \pm SEM (n = number of experiments). Cell numbers were separately counted in each experiment in one representative dish. Kinin concentrations measured in the homogenates were referred to 10^6 endothelial cells. Statistical significance was analysed by the *Mann-Whitney-U* test for unpaired samples. P values less than 0.05 were accepted as statistically significant.

Results

1. Immunostaining of tissue kallikrein

The presence of tissue kallikrein immunoreactivity was demonstrated by immunostaining. The endothelial cells from umbilical veins labelled with the anti-human kallikrein antibody appeared red; between 55 and 78% of cells showed a positive staining (n = 5, fig. 1). The staining of endothelial cells from human pulmonary arteries demonstrated tissue kallikrein immunoreactivity in 65–75% of the cells (n = 3). Control staining with serum instead of the specific antibody resulted in no specific phosphatase reactions. Between 85 and 95% of cells (n = 3) gave a positive staining reaction with factor VIII related antigen.

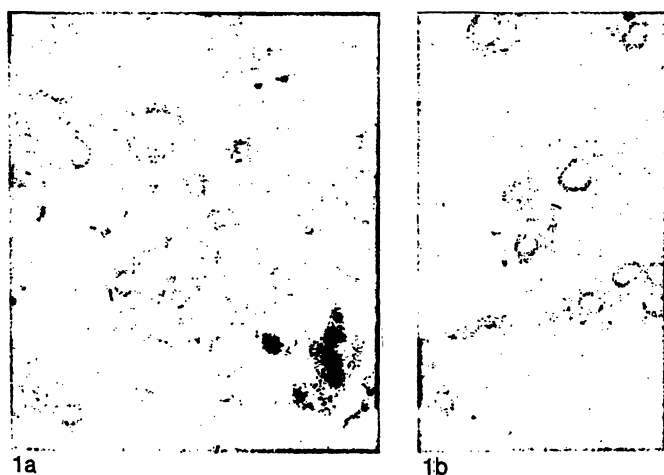


Fig. 1 Immunohistological detection of tissue kallikrein: a) light micrograph of endothelial cells from human umbilical veins stained with an anti-tissue kallikrein antibody; b) control staining without first antibody (Haematoxylin stain, magnification \times 110).

2. Enzymatic activity of tissue kallikrein in endothelial cells

Kallikrein enzyme activity in cell homogenates of endothelial cells from human umbilical veins was detected by the cleavage of *D*-Val-cyclohexyl-Ala-Arg-4-nitraniline. The total activity was determined as 5.0 ± 1.1 mU/ 10^6 cells (n = 8), the activity inhibited by aprotinin, attributed to tissue kallikrein-like activity, was 3.57 ± 0.47 mU/ 10^6 cells in endothelial cells from human umbilical veins. In endothelial cells from human pulmonary arteries the total activity was 11.2 ± 1.2 mU/ 10^6 cells, and the tissue kallikrein-like activity was 7.52 ± 0.82 mU/ 10^6 cells (n = 4, p < 0.01 vs. endothelial cells from human umbilical veins).

3. Kinin release into supernatants

In the presence of the angiotensin converting enzyme-inhibitor, lisinopril, and the neutral endopeptidase 24.11-inhibitor, phosphoramidon, the following kinin-concentrations were measured: 10 minutes 40 ± 18 ng/l, 1 hour 52 ± 23 ng/l, 4 hours 35 ± 15 ng/l (n = 9 at each time point). No immunoreactive kinins were detected in the incubation medium alone, or in supernatants without the peptidase inhibitors, at all time points (n = 6).

4. Kinin release in endothelial cells from human umbilical veins

No immunoreactive kinins were detected after homogenisation of cells in the absence of the peptidase inhibitors, lisinopril and phosphoramidon. In the presence of both inhibitors, kinin contents of 424 ± 83 pg/ 10^6 cells (n = 8) were measured directly after homogenisation. After 5 minutes, the kinin concentration increased further to 627 ± 57 pg/ 10^6 cells (n = 8) and remained nearly unchanged during 30 minutes. The presence of the tissue kallikrein inhibitor, aprotinin (1000 kIU/dish), resulted in significantly lower concentrations at all time points (P < 0.05 vs. control). Treatment of the cells with 100 mg/l dextran sulphate reduced the cellular kinin content from 424 ± 83 pg/ 10^6 cells to 102 ± 14 pg/ 10^6 cells (n = 8, p < 0.01). After 5 and 30 minutes, the kinin concentrations increased further to 150 ± 23 pg/ 10^6 cells (n = 8) and 186 ± 31 pg/ 10^6 cells (n = 8, p < 0.05 to 0 minutes; fig. 2a).

Addition of 5 mU tissue kallikrein immediately after homogenisation resulted in a fivefold increase during the first 5 minutes up to 2575 ± 400 pg/ 10^6 cells (n = 6, p < 0.01), which declined during the following 25 minutes (fig. 3).

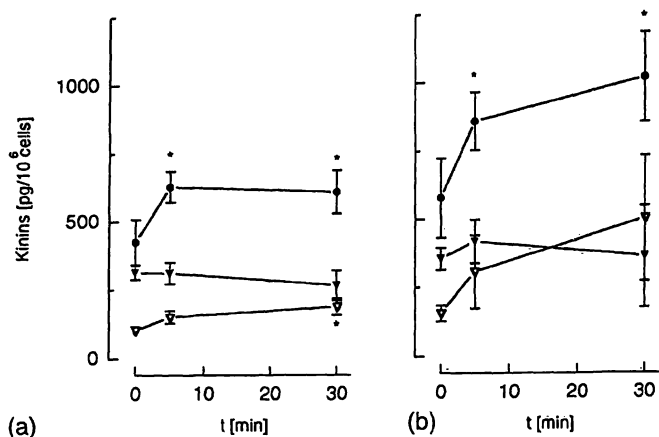


Fig. 2 Cellular kinin concentrations immediately after homogenisation and 5 and 30 minutes later in
 a) endothelial cells from human umbilical veins, and in
 b) endothelial cells from human pulmonary arteries.
 Controls (filled circles), endothelial cells treated with dextran sulphate (100 mg/l) before homogenisation (open triangles), and cells incubated with aprotinin (1000 kIU/dish, filled triangles * $P < 0.05$ vs. 0 minute).

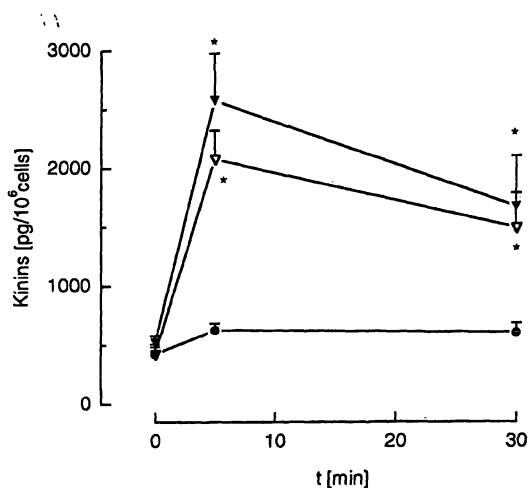


Fig. 3 Cellular kinin concentrations in endothelial cells from human umbilical veins immediately after homogenisation and 5 and 30 minutes after addition of 0.5 mU porcine kallikrein (open triangles) and 5 mU tissue kallikrein (filled triangles), compared with controls without kallikrein addition (filled circles) ($n = 9$ each * $P < 0.05$ vs. control).

5. Kinin release in endothelial cells from human pulmonary arteries

Similar experiments were performed with endothelial cells derived from the human pulmonary artery. In homogenates of endothelial cells from human pulmonary arteries, no immunoreactive kinins were detected without the peptidase inhibitors. In the presence of lisinopril and phosphoramidon, kinin contents of 576 ± 146 pg/10⁶ cells ($n = 4$) were detected immediately after homogenisation. After 5 and 30 minutes, the kinin concentration significantly increased to 857 ± 106 pg/10⁶ cells and 1117 ± 165 pg/10⁶ cells ($n = 4$, fig. 2, left panel), and was unchanged after 60 minutes (960 ± 60 pg/10⁶ cells, $n = 4$, not shown in fig. 2). As in endo-

thelial cells from human umbilical veins, the release of kinins was reduced in the presence of aprotinin. The kinin concentrations remained unchanged 30 minutes after homogenisation. Treatment of the cells with dextran sulphate significantly decreased cellular kinin concentrations (155 ± 28 pg/10⁶ cells, $n = 4$, $p < 0.05$). The kinin concentrations increased after 5 minutes to 305 ± 124 pg/10⁶ cells and after 30 minutes to 500 ± 245 pg/10⁶ cells ($n = 4$, fig. 2b).

Discussion

The present data provide immunohistological and functional evidence for the presence of tissue kallikrein in cultured endothelial cells from human umbilical veins and pulmonary arteries. About two thirds of the endothelial cells from umbilical veins and pulmonary arteries showed positive staining after immunostaining with a highly specific antiserum against tissue kallikrein (18). The control staining with the endothelial marker (factor VIII related antigen) was positive in nearly all the cells. This confirmed that the tissue kallikrein-immunoreactivity can be attributed to human endothelial cells. The tissue kallikrein antigen was not found in all the cells, possibly because low quantities of kallikrein are present, or the staining technique has low sensitivity. We observed a similar pattern of positive and negative cells in human smooth muscle cells (21). As reported previously by various authors (9–13), tissue kallikrein is present in vascular smooth muscle cells. Therefore, the apparent absence of immunoreactivity in some endothelial cells may be due to a methodological problem. In all experiments the majority of the endothelial cells were positive for tissue-kallikrein immunoreactivity.

Tissue kallikrein-like activity was measured by a well characterised colorimetric assay (19). Since the specificity of chromogenic substrates is not very high, we performed parallel measurements in the presence of the tissue kallikrein inhibitor, aprotinin, to exclude major unspecific protease activity, including plasma kallikrein. Aprotinin blocked more than 75% of the total enzymatic activity in endothelial cells. These results might indicate that the major kininogenase activity in endothelial cell can be attributed to a tissue kallikrein-like enzyme.

Kinins are generated by enzymatic cleavage of high molecular weight kininogen and low molecular weight kininogen by kallikreins. Both kininogens are synthesised in the liver and are found in plasma. High molecular mass kininogen binding sites have been detected on cultured endothelial cells (22, 23). A recently published study shows binding of high molecular mass kininogen to the endothelium of human umbilical veins in situ and the generation of bradykinin from the receptor-

bound high molecular weight kininogen after addition of plasma kallikrein (24). Therefore, it seems likely that high molecular weight kininogen is the major substrate of the endothelial kinin generation. *Van Iwaarden* et al. (22) reported that treatment of endothelial cells with dextran sulphate completely removed the receptor-bound high molecular weight kininogen. In our experiments, an approximately 75% reduction of kinin release in endothelial cells from human umbilical veins and endothelial cells from human pulmonary arteries occurred after washing with dextran sulphate. This indicates that the main source of kininogen is receptor-bound extracellular kininogen. Only the minor part of kininogen appears to be localized intracellularly. Whether intracellular kininogen is synthesised by endothelial cells or derives from serum or plasma is controversially discussed in various reports (22–24). In our study the intracellular kinin concentrations measured immediately after homogenisation were within the range of concentrations causing physiological responses like vasodilatation (1, 2, 6) and were 8 to 10 fold higher than the kinin concentrations found in the supernatants. We measured a further increase of kinin generation in endothelial cells during the following 30 minutes after homogenisation. The increase of kinin generation during this time can be explained by further cleavage of kininogen by cellular proteases, including kallikreins. Parallel incubation with the protease inhibitor, aprotinin, an inhibitor of tissue kallikrein, reduced cellular kinin concentrations and abolished the increase of kinins in the homogenates during this time period, suggesting a role for tissue kallikrein in endothelial kinin formation.

Since human umbilical vein endothelial cells are often considered to be embryonic cells, which might be different from adult phenotypes, we performed parallel experiments with adult endothelial cells derived from segments of the pulmonary artery. We obtained similar results for the measurement of tissue kallikrein-like activity and cellular kinin formation in endothelial cells from the pulmonary circulation, which confirms the results of the experiments with endothelial cells from human umbilical veins. Different amounts of kallikrein-like activity were measured in endothelial cells from hu-

man umbilical veins and endothelial cells from human pulmonary arteries. It has been reported before that tissue-like kallikrein activity varies in homogenates from different human vessels (12).

The present data demonstrate that certain requirements have to be met to measure kinin formation and release from endothelial cells. The most important condition is the inhibition of bradykinin degrading enzymes. The most potent of these is angiotensin converting enzyme (2). Another kininase II, the neutral endopeptidase 24.11, is also involved in the degradation of bradykinin (25). Intra- and extracellular kinins were only detected when both enzymes had been blocked by inhibitors. The second requirement is the exogenous supply of kininogen (22, 24), which is normally present in cell culture medium. Whether disintegration (like homogenisation) of the cells is a requirement for kinin release is not sure. However, we measured much higher concentrations of kinins in homogenates than in supernatants. Present data from different studies (6, 8, 25) indicate that physiological stimuli like flow, shear stress or ischaemia might be a stimulus for local kinin release.

A recent publication by *Wang* and colleagues provides evidence that tissue kallikrein is a powerful modulator of blood pressure in vivo (26). Transgenic mice carrying the human tissue kallikrein gene demonstrated hypotensive blood pressure, when tissue kallikrein was overexpressed in tissues and serum. Recent cloning of human tissue kallikrein gene and its transcripts from colon (27) might provide the basis for detailed investigation of tissue kallikrein expression in endothelial cells in the near future.

In the present study we demonstrated that tissue kallikrein is present in human endothelial cells derived from umbilical veins and pulmonary arteries, and that it contributes to local kinin formation in vitro.

Acknowledgement

The authors thank Mrs *U. Meisel-Rott* and Mrs *K. Vetter* for excellent technical assistance. The results were presented in part at the scientific sessions of the American Heart Association 1991.

References

- Roman, R. J., Kaldunski, M. L., Scicli, A. G. & Carretero, O. A. (1988) Influence of kinins and angiotensin II on the regulation of papillary blood flow. *Am. J. Physiol.* 255, F690–F698.
- Auch-Schwelk, W., Kuchenbuch, C., Claus, C., Walther, B., Bossaller, C., Friedel, N., Graf, K., Gräfe, M. & Fleck, E. (1993) Local regulation of vascular tone by bradykinin and angiotensin converting enzyme inhibitors. *Eur. Heart. J.* 14 (suppl. I), 154–160.
- Cherry, P. D., Furchgott, R. F., Zawadzki, T. V. & Jothianandan, D. (1982) Role of endothelial cells in relaxation of isolated arteries by bradykinin. *Proc. Natl. Acad. Sci. USA* 79, 105–110.
- McIntyre, T. M., Zimmerman, G. A., Satoh, K. & Prescott, S. M. (1985) Cultured endothelial cells synthesise both platelet activating factor and prostacyclin in response to histamine, bradykinin, and adenosine triphosphate. *J. Clin. Invest.* 76, 271–280.

5. Frey, E. K., Kraut, H. & Werle, E. (1977) *Kallikrein Padutin*. Stuttgart, Germany, Ferdinand Enke Verlag.
6. Wiemer, G., Schölkens, B. A., Becker, R. H. A. & Busse, R. (1991) Ramiprilat enhances endothelial autacoid formation by inhibiting breakdown of endothelium-derived bradykinin. *Hypertension* 18, 558–563.
7. Busse, R. & Lamontagne, D. (1991) Endothelium-derived bradykinins is responsible for the increase in calcium produced by angiotensin-converting enzyme inhibitors in human endothelial cells. *Naunyn. Schmiedebergs Arch. Pharmacol.* 344, 126–129.
8. Baumgarten, C. R., Linz, W., Kunkel, G., Schölkens, B. A. & Wiemer, G. (1993) Ramiprilat increases bradykinin outflow from isolated rat hearts. *Br. J. Pharmacol.* 108, 293–295.
9. Oza, N. B., Schwartz, J. H., Goud, H. D. & Levinsky, N. G. (1990) Rat aortic smooth muscle cells in culture express kallikrein, kininogen, and bradykinase activity. *J. Clin. Invest.* 85, 597–600.
10. Nolly, H., Scicli, A. G., Scicli, G. & Carretero, O. A. (1985) Characterisation of a kininogenase from rat vascular tissue resembling glandular kallikrein. *Circ. Res.* 56, 816–821.
11. Saed, G. M., Carreteto, O. A., MacDonald, R. J. & Scicli, A. G. (1990) Kallikrein messenger RNA in rat arteries and veins. *Circ. Res.* 67, 510–516.
12. Madeddu, P., Gherli, T., Bacciu, P. B., Maioli, M. & Glorioso, N. (1993) A kallikrein-like enzyme in human vascular tissue. *Am. J. Hypertens.* 6, 344–348.
13. Moshi, M. J., Zeitlin, I. J., Wainwright, C. L. & Parrat, J. R. (1992) Acid optimum kininogenases in canine myocardium and aorta. *Cardiovasc. Res.* 26, 367–370.
14. Jaffe, E. A., Nachman, R. L., Becker, C. G. & Minick, C. R. (1973) Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria. *J. Clin. Invest.* 52, 2745–2756.
15. Gräfe, M., Bossaller, C., Graf, K., Auch-Schwelk, W., Baumgarten, C. R., Hildebrand, A. & Fleck, E. (1993) Effect of angiotensin converting enzyme inhibition on bradykinin metabolism by human vascular endothelial cells. *Am. J. Physiol.* 264, H1493–H1497.
16. Voyta, J. C., Via, D. P., Butterfield, C. E. & Zetter, B. R. (1984) Identification and isolation of endothelial cells based on their increased uptake of acetylated-low density lipoprotein. *J. Cell. Biol.* 99, 2034–2040.
17. Cordell, J., Falini, B., Erber, O. N., Gosh, A. K., Abdulaziz, Z., MacDonald, S., Polford, K., Stein, H. & Mason, D. Y. (1984) Immunoenzymatic labelling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). *J. Histochem. Cytochem.* 32, 219–227.
18. Baumgarten, C. R., Schwarting, R. & Kunkel, G. (1989) Localisation of glandular kallikrein in nasal mucosa of allergic and non allergic individuals. *Adv. Exp. Med. Biol.* 247B, 523–528.
19. Amundsen, E., Potter, J., Friberger, P., Knos, M., Larsbraten, M. & Claeson, G. (1979) Methods for determination of glandular kallikrein by means of a chromogenic tripeptide substrate. *Adv. Exp. Med. Biol.* 120a, 83–95.
20. Proud, D., Togias, A., Naclerio, R. M., Crush, S. A., Norman, P. S. & Liechtenstein, L. M. (1983) Kinins are generated in vivo following nasal airway challenge of allergic individuals with allergen. *J. Clin. Invest.* 72, 1678–1685.
21. Graf, K., Gräfe, M., Bossaller, C., Baumgarten, C. R., Auch-Schwelk, W. & Fleck, E. (1991) Kininogenase augments bradykinin concentrations in endothelial and smooth muscle cells in culture. *Circulation* 84 (suppl.), 624.
22. Van Iwaarden, F., de Groot, P. G., Sixma, J. J. & Bouma, B. N. (1988) High-molecular weight kininogen is present in cultured human endothelial cells: Localisation, isolation and characterisation. *Blood* 71, 1268–1276.
23. Schmaier, A. H., Kuo, A., Lundberg, D., Murray, S. & Cines, D. (1988) The expression of high molecular weight kininogen on human umbilical vein endothelial cells. *J. Biol. Chem.* 263, 16327–16333.
24. Graf, K., Gräfe, M., Bossaller, C., Niehus, J., Schulz, K. D., Auch-Schwelk, W. & Fleck, E. (1993) Degradation of bradykinin by neutral endopeptidase 24.11 in cultured human endothelial cells. *Eur. J. Clin. Chem. Clin. Biochem.* 31, 267–272.
25. Nishikawa, K., Shibayama, Y., Kuna, P., Calcaterra, E., Kaplan, A. P. & Reddigari, S. R. (1992) Generation of vasoactive peptide bradykinin from human umbilical vein endothelium bound high molecular weight kininogen by plasma kallikrein. *Blood* 80, 1980–1988.
26. Wang, J., Xiong, W., Yang, Z., Davis, T., Dewey, M. J., Chao, J. & Chao, L. (1994) Human tissue kallikrein induces hypotension in transgenic mice. *Hypertension* 23, 236–243.
27. Chen, L.-M., Murray, S. R., Chai, K. X., Richards, G. P., Chao, J. & Chao, L. (1993) Molecular cloning and characterization of human tissue kallikrein and a novel kallikrein transcripts in colon. *Kinin* 93, p. 17.

Dr. med. Kristof Graf
 German Heart Institute Berlin
 Department of Internal Medicine and Cardiology
 Augustenburger Platz 1
 D-13353 Berlin
 Germany