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The Hydrolysis of Biologically Active Peptides by Bovine Lung Tissue Factor (Thromboplastin)[1] (39444)

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Tissue extracts from almost all mammalian organs contain tissue factor activity which can promote blood coagulation via the extrinsic system (1). Nemerson and Pitlick (2) solubilized tissue factor from bovine lung and brain and purified the protein component. Delipidation and solubilization resulted in a loss of coagulant activity which could be restored by recombining the apoprotein with appropriate phospholipids (2, 3). Pitlick *et al.* (4) observed that particulate as well as delipidated tissue factor possessed peptidase activity. Tissue factor could hydrolyze synthetic di- and tripeptides and aminoacyl-β-naphthylamides and release a number of free amino acids from the oxidized B chain of insulin.

It was of interest to determine if tissue factor peptidase activity might serve the function of metabolizing physiologically important peptides. This paper presents data demonstrating the ability of relipidated and apoprotein tissue factor from bovine lung to hydrolyze a number of vasoactive peptides and hypothalamic and neurohypophyseal hormones. Preliminary reports have appeared elsewhere (5, 6).

Materials. Dansyl chloride and dansyl amino acids were purchased from Sigma Chemical Co. Additional dansyl amino acid and peptide markers were prepared from the respective amino acids and peptides by the method of Gray (7). Polyamide layer sheets, prepared by the Cheng Chin Trading Co., were purchased through Gallard-Schlesinger. Synthetic peptides were obtained as follows: substance P (Beckman Instruments, Spinco Division); oxytocin (A grade, Calbiochem); bradykinin, Lys-bradykinin, Met-Lys-bradykinin, [Asp[1],Ile[5]]-angiotensin I, [Asp[1],Ile[5]]-angiotensin II, bradykinin potentiating pentapeptide (BPP_{5a})[3] and nonapeptide (BPP_{9a}) from *Bothrops jararaca*, LH-RH, TRF, and human fibrinopeptide A (Spectrum Medical Industries); MIF and additional quantities of LH-RH and oxytocin (Dr. Maurice Manning, Medical College of Ohio at Toledo); [Sar[1],Ile[5]]-angiotensin II and [Me_2Gly[1],-Ile[5]]-angiotensin II (Dr. M. C. Khosla, Cleveland Clinic Foundation). All peptides were assayed for purity, for amino-terminal residues, and for amino acid composition by the dansylation method (7). Additional materials were obtained as follows: ninhydrin (Schwarz/Mann); constant-boiling hydrochloric acid (Pierce Chemical); 2-mercaptoethanol (Eastman). All other chemicals were reagent grade.

Mixed brain phospholipids were prepared essentially in the manner described by Nemerson (8). Micellization (3) was performed in 0.5% sodium deoxycholate.

Tissue factor purification. Tissue factor apoprotein was purified from bovine lung by the method of Nemerson and Pitlick (2), using hydrolysis of alanyl-β-naphthylamide as the enzyme assay (4). Disc gel electro-

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[3] Abbreviations: LH-RH, luteinizing hormone-releasing hormone; MIF, melanocyte stimulating hormone-release-inhibiting factor (Pro-Leu-Gly-NH_2); TRF, thyrotropin-releasing factor; [Sar[1]]-angiotensin II, [1-*N*-methylglycine]-angiotensin II; [Me_2Gly[1]]-angiotensin II, [1-*N*,*N*-dimethylglycine]-angiotensin II; BPP_{5a}, bradykinin potentiating peptide (pentapeptide from *Bothrops jararaca*); BPP_{9a}, bradykinin potentiating peptide (nonapeptide from *B. jararaca*); DNS-, dansyl (5-dimethylaminonaphthalene-1-sulfonyl).

phoresis of purified tissue factor apoprotein on standard gels (9) revealed a single major protein band which barely entered the separation gel and a minor contaminant running near the front.

Relipidation of tissue factor apoprotein. Purified tissue factor apoprotein was relipidated with mixed brain phospholipids according to Nemerson (10) at a ratio of 5.0 mg of lipid/mg of protein. Relipidated tissue factor contained coagulant activity as measured with the one-stage prothrombin time assay (11, 12) while apoprotein was completely devoid of coagulant activity.

Peptidase activity. Relipidated tissue factor and tissue factor apoprotein control were assayed for their ability to hydrolyze a series of physiologically active peptides by measuring the increase in the appearance of free amino groups with time by the ninhydrin method (13). Assay mixtures consisted of 0.05 ml of enzyme solution (15 μg protein) and 0.1 ml of 0.5 m*M* substrate in 0.02 *M* potassium phosphate, pH 6.8. The mixtures were incubated at 37° and 25-μl aliquots were removed at 1 min and 2.5, 5.0, and 7.5 hr and inactivated with 1 ml of ninhydrin.

Dansylation and chromatography of tissue factor–peptide incubation mixtures. Tissue factor-peptide incubation mixtures were dansylated by the method of Gray (7) and chromatographed in order to identify reaction products. Relipidated tissue factor and tissue factor apoprotein (15 μg in 0.05 ml) were incubated at 37° with 0.1 ml of 0.5 m*M* substrate in 0.02 *M* potassium phosphate, pH 6.8. Aliquots (10 μl) were removed from the incubation mixtures at 1 min and 2.5, 5.0, and 7.5 hr and placed in 15 μl of 0.2 *M* sodium bicarbonate to which was immediately added 25 μl of dansyl chloride reagent (5 mg/ml in acetone). After completion of the dansylation reaction (1 hr at 37°), samples were dried *in vacuo* and then resuspended in 25 μl of acetone–0.1 *N* acetic acid (3:2). A portion of each dansylated reaction mixture (1.5 μl) was chromatographed in two dimensions on 7.5 × 7.5-cm polyamide layer sheets using Solvent I (water–90% formic acid, 200:3) and Solvent II (benzene–glacial acetic acid, 9:1).

A second portion of each dansylated mixture was acid hydrolyzed by heating the sample with constant-boiling HCl in a sealed tube at 105° for 9–16 hr. After removal of the HCl *in vacuo* over NaOH pellets, samples were resuspended and chromatographed on polyamide layers as described above.

Fluorescent spots were detected on chromatograms with an ultraviolet lamp at 254 nm and identified by comparing R_f values with R_f values of standard dansyl amino acids and peptides chromatographed separately in each solvent. In most cases, the identities of fluorescent spots were confirmed by chromatographing samples containing selected internal dansyl amino acid standards. The individual synthetic peptide substrates and the enzyme solutions were dansylated separately and chromatographed, both with and without prior acid hydrolysis. These chromatograms served as controls. Qualitative amino acid analysis of each peptide was determined by chromatographing a sample of the peptide which had first been acid hydrolyzed and then dansylated.

Chemical methods. Protein concentration was determined by the method of Lowry *et al.* (14). The phosphorous content of mixed brain phospholipids was determined by the method of Chen *et al.* (15).

Results. Effect of tissue factor on physiologically active peptides. Figure 1 shows that both relipidated and apoprotein tissue factor extensively hydrolyzed bradykinin, Lys-bradykinin, Met-Lys-bradykinin, substance P, angiotensin I, angiotensin II, and fibrinopeptide A, while acting more slowly on [Sar^1]-angiotensin II, [Me_2Gly^1]-angiotensin II, and bradykinin potentiating pentapeptide from *B. jararaca* (BPP_{5a}). LH-RH, MIF, and oxytocin were also hydrolyzed quite slowly while TRF and bradykinin potentiating nonapeptide from *B. jararaca* (BPP_{9a}) were not hydrolyzed at all. There was no difference in the rate of hydrolysis of any of the peptides by either relipidated tissue factor or tissue factor apoprotein under the conditions of our assay. Dansylated enzyme–peptide reaction mixtures chromatographed after various times of incubation indicated that relipidated tissue factor and tissue factor apoprotein degraded peptides by identical mechanisms.

Bradykinin. Figure 1a shows that 50

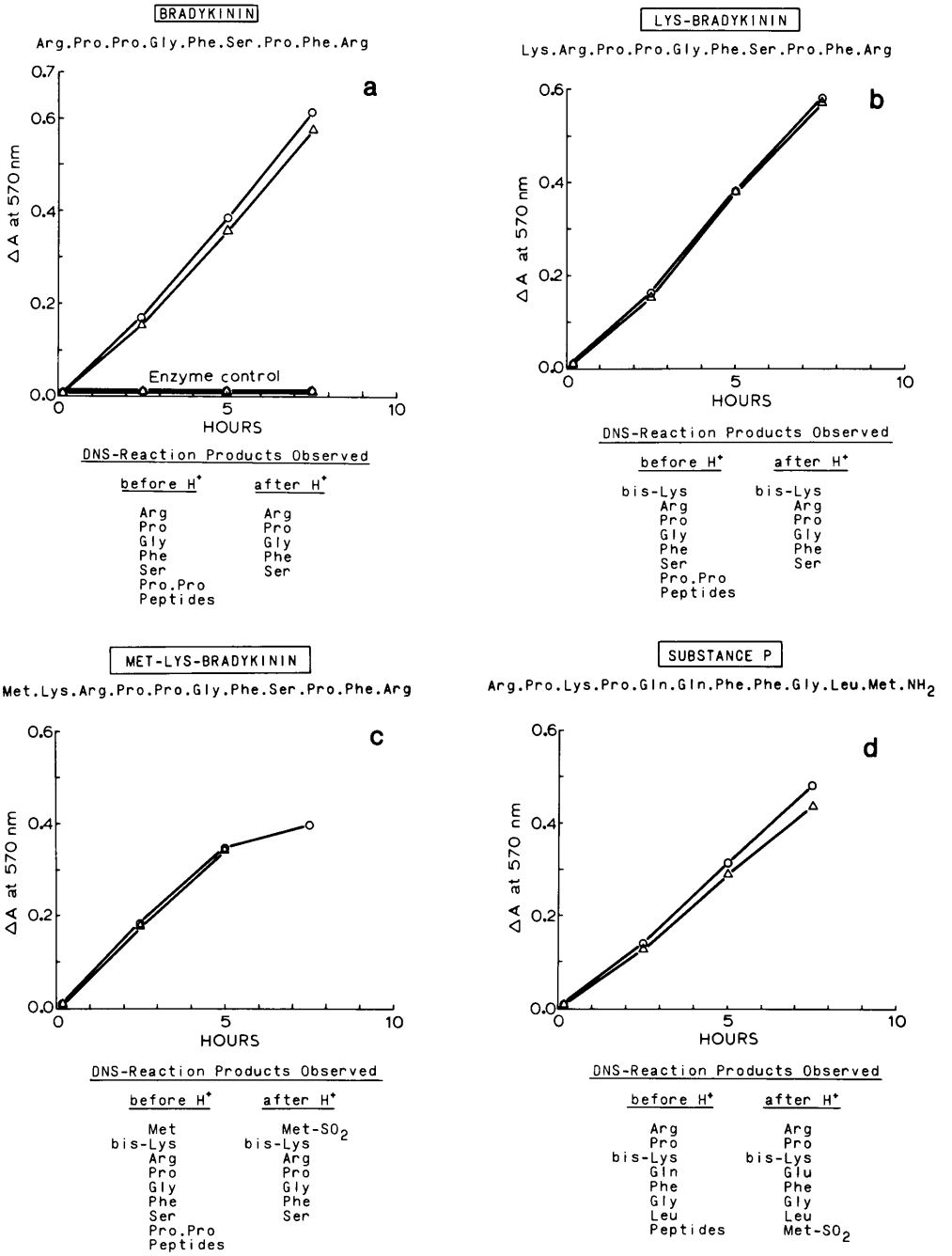

FIG. 1. The effect of relipidated and apoprotein bovine lung tissue factor on physiologically active peptides. Relipidated tissue factor or tissue factor apoprotein (15 μg in 0.05 ml) was incubated at 37° with 0.1 ml of 0.5 m*M* substrate in 0.02 *M* potassium phosphate, pH 6.8. Aliquots (25 μl) were removed at 1 min and 2.5, 5.0, and 7.5 hr and inactivated with 1 ml of ninhydrin. The ninhydrin tubes were developed by heating in a boiling-water bath for 20 min. After dilution with 1 ml of 1-propanol-H_2O (1:1), samples were read at 570 nm in a Turner Model 330 spectrophotometer with an Instafill attachment. The absorbance at 1 min was subtracted from the values at other time periods. Graphs in the figure indicate ΔA as a function of incubation time (hours). A ΔA = 0.097 represents 50 nmoles of free amino groups liberated from the 50 nmoles of substrate (based on the color yield of leucine), indicating approximately one bond cleaved. Δ, Relipidated; O, apoprotein. Reaction products were identified by dansylating aliquots of incubation mixtures by the method of Gray (7) at the different time periods given above. The dansylated mixtures were chromatographed on polyamide layer sheets either before acid hydrolysis (before H^+) or else after acid hydrolysis to release terminal DNS-amino acids from peptides (after H^+). The figure indicates those dansyl reaction products observed after 7.5 hr. The products were identical for both relipidated and apoprotein tissue factor. Bis- indicates that the side chain functional group as well as the α-amino group of the amino acid was dansylated. α-His and ϵ-Lys indicate that only the α-amino group of histidine and the ϵ-amino group of lysine, respectively, were dansylated.

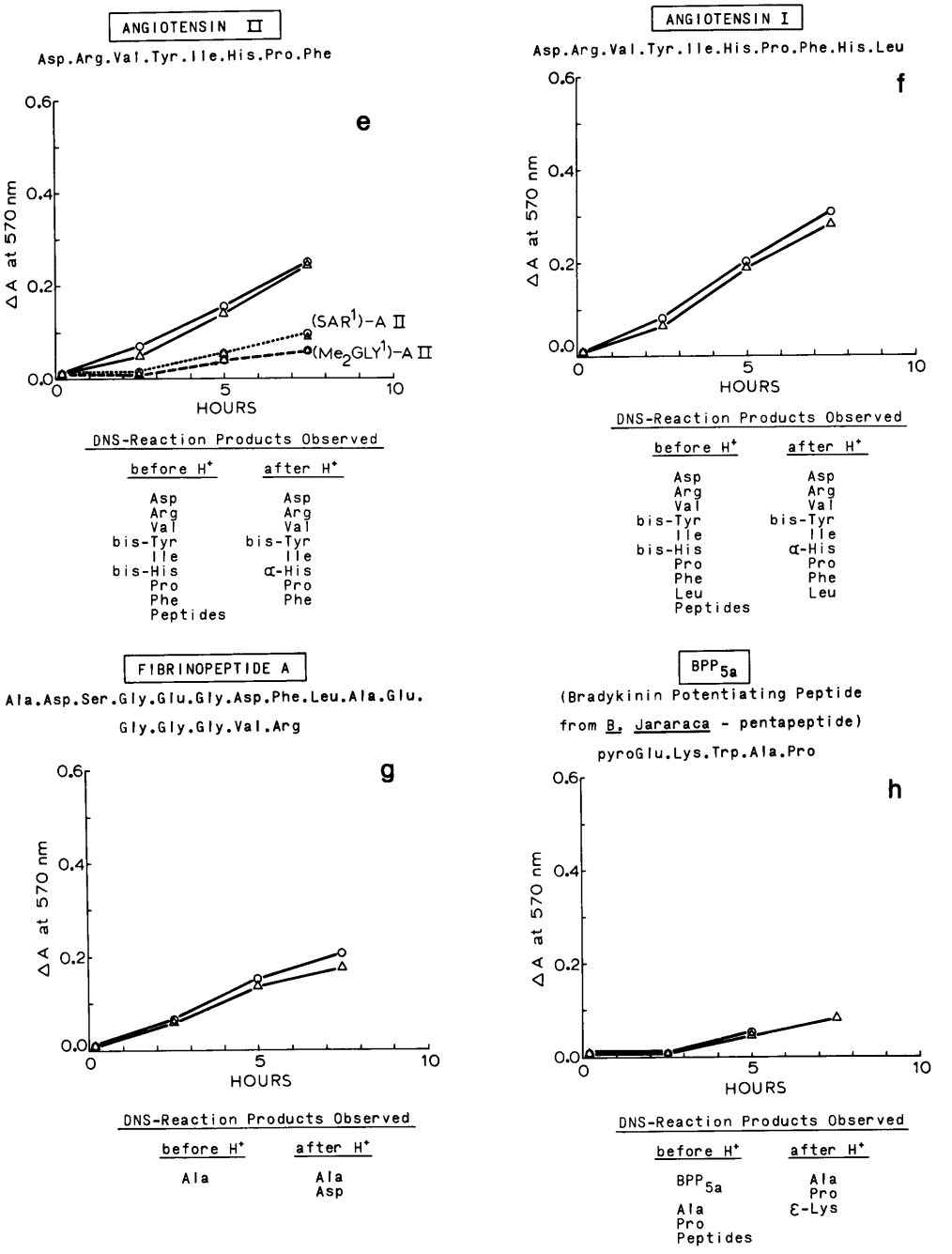

FIGURE 1(e-h).

nmoles of bradykinin was extensively hydrolyzed by 15 μg of either relipidated or apoprotein tissue factor as measured by the ninhydrin method. Both enzyme preparations, when incubated alone, showed no increase in ninhydrin color. The dansylated enzyme-bradykinin incubation mixtures, which were acid hydrolyzed to release terminal DNS-amino acids from intermediate peptides and then chromatographed, also indicated extensive degradation of the peptide. Since each dansyl amino acid observed indicated

hydrolysis of the peptide bond on the amino side of that residue, it was concluded that tissue factor hydrolyzed bradykinin at the Pro^3Gly^4 and Phe^5Ser^6 bonds and at the amino side of at least one of the three prolines and at least one of the two phenylalanines. The intensity of the DNS-Arg spot increased with increasing incubation time suggesting that, in addition to the constant level of DNS-Arg contributed by the N-terminal residue, DNS-Arg was also being contributed by arginine released from the C terminus. Thus, tissue factor also hydrolyzed the Phe^8Arg^9 bond. Chromatograms of nonacid-hydrolyzed, dansylated incubation mixtures, which indicated the actual composition of the reaction mixtures, revealed several spots having R_f values identical to several free dansyl amino acids. The presence of free DNS-Gly and DNS-Ser in the mixture suggested that the Gly^4Phe^5 and Ser^6Phe^7 bonds were also hydrolyzed. Intermediate DNS-peptides were also observed. One peptide had an N-terminal DNS-Pro and cochromatographed with authentic DNS-Pro-Pro. This indicated that tissue factor hydrolyzed the Arg^1Pro^2 bond. A second peptide had an N-terminal DNS-Ser. The intensities of free DNS-Ser and DNS-Pro spots did not increase as rapidly with time as other free DNS-amino acids confirming that tissue factor hydrolyzed the Ser^6Pro^7 and Pro^2Pro^3 bonds more slowly than other bonds in the molecule. In summary, no fewer than six bonds were cleaved in bradykinin.

Lys-bradykinin. Figure 1b shows that relipidated and apoprotein tissue factor hydrolyzed Lys-bradykinin at about the same rate as bradykinin. Dansyl chromatograms indicated that the mechanism of degradation of Lys-bradykinin was also similar to that of bradykinin. The chromatograms of the nonacid-hydrolyzed dansylated reaction mixtures revealed a spot with R_f values identical to free bis-DNS-Lys, suggesting that tissue factor also hydrolyzed the Lys^1Arg^2 bond.

Met-Lys-bradykinin. Met-Lys-bradykinin was hydrolyzed by tissue factor at a rate and by a mechanism similar to bradykinin and Lys-bradykinin (Fig. 1c). In this case, the Met^1Lys^2 bond was also hydrolyzed.

Substance P. Figure 1d indicates that tissue factor extensively hydrolyzed substance P, potentially cleaving every peptide bond in the molecule. Chromatograms of dansylated reaction mixtures at different periods of incubation suggested that the Pro^2Lys^3, Gln^5Gln^6, Gln^6Phe^7, and Phe^7Phe^8 bonds were hydrolyzed rapidly while Arg^1Pro^2, Lys^3Pro^4, Phe^8Gly^9, and $Leu^{10}Met^{11}$ bonds were hydrolyzed more slowly. The Gly^9Leu^{10} bond was cleaved at an intermediate rate and the Met-NH_2 amide bond was not hydrolyzed at all. It was impossible from the present data to determine whether or not the Pro^4Gln^5 bond was cleaved.

Angiotensin II. [Asp^1Ile^5]-angiotensin II was hydrolyzed by tissue factor at a rate less than one-half that of bradykinin (Fig. 1e). However, dansyl chromatograms indicated that tissue factor could degrade angiotensin II to constituent amino acids. The chromatograms of the nonacid-hydrolyzed dansylated mixtures revealed the presence of a major intermediate peptide which had a terminal DNS-His. The slow appearance of free DNS-His and DNS-Pro confirmed that the His^6Pro^7 bond was more resistant to cleavage than other bonds in the molecule.

[Sar^1]-angiotensin II and [Me_2Gly^1]-angiotensin II. Figure 1e also shows that [Sar^1]-angiotensin II and [Me_2Gly^1]-angiotensin II were hydrolyzed at rates 40 and 25%, respectively, as fast as [Asp^1]-angiotensin II. Dansyl chromatographic data indicated that major cleavage in these analogs occurred at Pro^7Phe^8 with the release of free phenylalanine. Although the remainder of the molecule could be degraded to constituent amino acids, the rate was greatly retarded compared to [Asp^1]-angiotensin II.

Angiotensin I. Angiotensin I was hydrolyzed by tissue factor at a rate slightly greater than angiotensin II and was also cleaved at every peptide bond (Fig. 1f). The chromatograms of the non-acid-hydrolyzed dansylated reaction mixtures revealed two major intermediate peptides, both of which had N-terminal DNS-His residues. One of the peptides had the same R_f value as a similar spot in angiotensin II chromatograms and was presumably His-Pro. The other peptide was seen only in angiotensin I chromatograms and was probably His-Leu. The slow appearance of free DNS-His, DNS-Pro, and DNS-Leu confirmed these

assignments and indicated that the His^6Pro^7 and His^9Leu^{10} bonds were more resistant to cleavage than other bonds in the molecule. There was no evidence to suggest, however, that tissue factor initially released His-Leu to form angiotensin II before further degrading the active portion of the molecule.

Human fibrinopeptide A. Figure 1g shows that tissue factor hydrolyzed fibrinopeptide A but at a rate which suggested only limited cleavage of this 16-amino-acid peptide. Chromatograms of acid-hydrolyzed dansylated reaction mixtures revealed the presence of DNS-Asp, suggesting cleavage of either the Ala^1Asp^2 or Gly^6Asp^7 bond or both. The observed DNS-Ala spot came from the N terminus of fibrinopeptide A. This spot did not increase in intensity with increasing incubation time indicating that the Leu^9Ala^{10} bond was not hydrolyzed.

Chromatograms of nonacid-hydrolyzed dansylated samples revealed only DNS-Ala, suggesting that alanine was released from the N terminus. Thus, major cleavage of fibrinopeptide A by tissue factor occurred at the Ala^1Asp^2 bond, although the possibility of additional cleavage at Gly^6Asp^7 could not be eliminated.

Bradykinin potentiating pentapeptide from B. jararaca (BPP_{5a}). Figure 1h shows that tissue factor hydrolyzed BPP_{5a} only slowly. Dansyl chromatograms suggested that proline and alanine were released from the C terminus. There was considerably more DNS-Ala than DNS-Pro in the acid-hydrolyzed dansylated samples, suggesting that initial cleavage may have taken place at Trp^3Ala^4 followed by slower hydrolysis of the resulting dipeptide, Ala-Pro. The nonacid-hydrolyzed samples revealed the slow but equal appearance of free DNS-Ala and DNS-Pro confirming this conclusion. There was some evidence that tryptophan could be released from BPP_{5a} upon extended incubation with tissue factor.

Bradykinin potentiating nonapeptide from B. jararaca (BPP_{9a}) (pyroGlu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro). Tissue factor did not hydrolyze BPP_{9a}. Dansyl chromatograms revealed no detectable hydrolysis products.

LH-RH (pyroGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2). Tissue factor hydrolyzed LH-RH slowly. Dansyl chromatograms revealed several faint spots which have not yet been firmly identified.

MIF (Pro-Leu-Gly-NH_2). MIF was also hydrolyzed quite slowly by tissue factor. However, dansyl chromatograms indicated that tissue factor did hydrolyze both peptide bonds and the terminal amide bond. Chromatograms of nonacid-hydrolyzed dansylated reaction mixtures indicated that DNS-MIF was by far the most intense spot, confirming the ninhydrin data. DNS-Pro, DNS-Leu, and DNS-Gly-NH_2 were all faint but of equal intensity. This suggested that hydrolysis of one of the peptide bonds occurred with difficulty followed by immediate hydrolysis of the second bond. Hydrolysis of the C-terminal amide bond occurred only after extended periods of incubation.

Oxytocin (Cy_s-Tyr-Ile-Gln-Asn-Cy_s-Pro-Leu-Gly-NH_2). Oxytocin was hydrolyzed very slowly by tissue factor. Dansyl chromatograms revealed a variety of faint spots indicating that hydrolysis of oxytocin was possible, albeit with difficulty.

TRF (pyroGlu-His-Pro-NH_2). Tissue factor did not hydrolyze TRF. Dansyl chromatograms revealed no hydrolysis products.

Effect of 2-mercaptoethanol on relipidated tissue factor hydrolysis of bradykinin. Figure 2 shows that relipidated tissue factor hydrolysis of bradykinin was reduced about 90% in the presence of 20 m*M* 2-mercaptoethanol as measured by the ninhydrin method. Tissue factor apoprotein hydrolysis of bra-

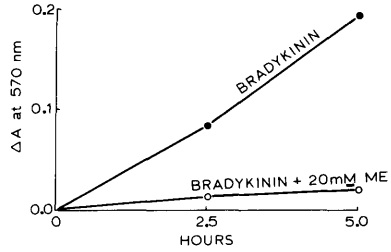

FIG. 2. Effect of 2-mercaptoethanol on the hydrolysis of bradykinin by relipidated tissue factor. Assay mixtures consisted of 0.05 ml relipidated tissue factor (12 μg protein), 0.05 ml of 0.05 m*M* bradykinin in 0.02 *M* potassium phosphate, pH 6.8, and 0.05 ml of 60 m*M* 2-mercaptoethanol in the same buffer (final concentration of 2-mercaptoethanol was 20 m*M*). A control was run in which the 2-mercaptoethanol was replaced by buffer. Mixtures were incubated at 37° and 25 μl aliquots were removed at 1 min., 2.5 hr, and 5 hr and assayed by the ninhydrin method as described in Fig. 1.

dykinin and angiotensin I was also reduced by about 80 and 90%, respectively, in the presence of 20 m*M* 2-mercaptoethanol.

Discussion. The data presented here extend the observations of Pitlick *et al.* (4) by demonstrating that the peptidase activity associated with bovine lung tissue factor can degrade a series of physiologically significant peptides. Since tissue factor represents approximately 1% of the particulate and nonextractable protein of bovine lung (2), the broad specificity of this enzyme toward peptide substrates may implicate it along with other peptidases (16) in a variety of physiological activities other than those related to direct initiation of the extrinsic clotting mechanism.

Tissue factor can readily hydrolyze the kinins and angiotensins *in vitro*. Ryan and co-workers (17–21) reported earlier that bradykinin and angiotensin I are hydrolyzed in the isolated perfused rat lung by enzymes which are probably located near the luminal surface of vascular endothelial cells. Furthermore, the pattern of hydrolysis of peptide bonds of bradykinin and its higher homologs by perfused lung enzymes is essentially identical to that reported for tissue factor herein. Zeldis *et al.* (22), using peroxidase-conjugated antibodies raised against purified tissue factor apoprotein, found that the antigen was located in highest concentration on the plasma membranes of vascular endothelial cells from all tissues studied, including lung. Inasmuch as tissue factor can hydrolyze the kinins and angiotensins *in vitro* and is known to be located at the site of hydrolysis *in vivo,* it is suggested that tissue factor may be one of the enzymes involved in the metabolism of circulating vasoactive peptides.

Although angiotensin-converting enzyme (kininase II) has been implicated in pulmonary metabolism of circulating kinins (23), the known specificity of this enzyme cannot account for all of the cleavages seen in the bradykinin molecule upon passage through the isolated lung. Furthermore, Stewart *et al.* (24) showed that BPP_{5a}, an inhibitor of angiotensin-converting enzyme, could only reduce the pulmonary inactivation of bradykinin in the rat from 98% to a plateau level of 30%. The ability of lung to inactivate 30% of intravenously injected bradykinin in the presence of large doses of BPP_{5a} suggests the presence of another inactivating enzyme, possibly tissue factor. Tissue factor was observed in this study to be greatly inhibited by 2-mercaptoethanol. Similarly, 2-mercaptoethanol destroyed all remaining bradykininase activity in lung and actually potentiated the effect of BPP_{5a}, perhaps by inhibiting its destruction by tissue factor. It is noteworthy that although BPP_{5a} is a more potent inhibitor of angiotensin-converting enzyme than BPP_{9a} *in vitro* (25), it is not as potent *in vivo,* having a much shorter half-life (26). Correspondingly, tissue factor can slowly destroy BPP_{5a}, but leaves BPP_{9a} intact even after a 7.5-hr incubation.

Tissue factor was observed to degrade angiotensin I and II to constituent amino acids. Ryan *et al.* (19, 20) observed that perfused rat lung also hydrolyzed angiotensin I at the His^6Pro^7, Pro^7Phe^8, and Phe^8His^9 bonds and possibly at the Tyr^4Ile^5 and Ile^5His^6 bonds. Additional cleavages probably occurred at the amino terminal end (19) but were not identified. Recently Ryan indicated (27) that rat lung also destroyed 10% of perfused angiotensin II, even though the perfusate showed no reduction in blood pressure response. Although angiotensin-converting enzyme was, undoubtedly, at least partially responsible for cleavage of the Phe^8His^9 bond of angiotensin I, it could not have hydrolyzed any of the remaining bonds in angiotensin I or II (28). It is therefore suggested that tissue factor may have been involved in the degradation of these peptides. The fact that tissue factor could hydrolyze the His^9Leu^{10} bond only slowly is consistent with perfusion studies with [10-*Leu*-^{14}C]-angiotensin I which indicated no hydrolysis of this bond by rat lung in one study (19) and only 3% hydrolysis in another study (23). In this regard, it might be noted that tissue factor could contribute to "angiotensin-converting enzyme" activity in crude tissue preparations when activity is measured by the rate of release of His-Leu from angiotensin I.

Tissue factor extensively degraded substance P. Boileau *et al.* (29) reported that substance P was inactivated 12% upon a single passage through the pulmonary circulation of the rat. Since angiotensin-convert-

ing enzyme would not be expected to hydrolyze substance P because of its C-terminal amide bond (30), the observed disappearance of this peptide in lung may be due to hydrolysis by tissue factor.

It was previously suggested (4) that tissue factor may be similar or identical to other particle-bound peptidases which have been studied in other organs (31–36). Indeed, discovery of the ubiquitous nature of tissue factor (22) suggests that this enzyme may be at least partially responsible for the destruction of vasoactive peptides reported for several perfused vascular beds other than lung (37, 38) and for homogenate and particulate fractions from a variety of tissues (27, 31, 39–45).

Recent evidence has suggested that tissue factor may be available to hydrolyze circulating peptides without at the same time initiating blood coagulation. Maynard *et al.* (46) have shown that tissue factor exists on the outside of plasma membranes of cultured WISH amnion cells and human skin fibroblasts. Yet, tissue factor coagulant activity is masked, possibly by the cell surface coat. Thus, it is possible that circulating peptides could penetrate the surface coat of vascular endothelial cells and interact with tissue factor, whereas the large protein molecule, factor VII, could not. Another possibility is based on the suggestion by Smith and Ryan (47) that enzymes which hydrolyze circulating peptides may be located within caveolae intracellulares which are open to the vascular lumen but covered with a delicate diaphragm, as is the case for 5′-nucleotidase. If tissue factor is located within these structures, it is possible that the diaphragm may be permeable to peptides but impermeable to circulating factor VII and other clotting factors. Disruption of the diaphragm during tissue damage may be necessary to expose tissue factor and initiate coagulation.

The present investigators eliminated the possibility that tissue factor used in these studies is contaminated with pancreatic-type carboxypeptidase B; catheptic carboxypeptidase B, and plasma carboxypeptidase N (all kininases) since these enzymes would be expected to release the C-terminal arginine from human fibrinopeptide A (48). The fact that tissue factor is active at neutral pH (4), is sensitive to thiol-reducing agents, but is not affected by DFP, soybean trypsin inhibitor or Ca^{2+} (4) suggests that this preparation is not contaminated by some of the several known kininases or angiotensinases (27, 48). Furthermore, there is no effect of Cl^- on tissue factor up to 0.1 *M* using Leu-Gly-Gly as substrate, suggesting that the preparation does not contain angiotensin-converting enzyme (28). Clotting and peptidase activity, however, remain coincident for the extensively purified relipidated enzyme (4).

The ability of tissue factor to degrade physiologically active peptides suggests important functions for this enzyme in addition to those related to blood coagulation. Recognition of the full significance of tissue factor peptidase activity *in vivo* may depend on the discovery of a highly specific inhibitor which will enable investigators to detect participation of tissue factor in various physiological mechanisms.

Summary. Tissue factor apoprotein and relipidated tissue factor preparations extensively hydrolyze bradykinin, Lys-bradykinin, Met-Lys-bradykinin, substance P, [Asp^1,Ile^5]-angiotensin II, [Asp^1,Ile^5]-angiotensin I, and human fibrinopeptide A while acting more slowly on [Sar^1,Ile^5]-angiotensin II, [Me_2Gly^1,Ile^5]-angiotensin II, bradykinin potentiating pentapeptide from *B. jararaca,* luteinizing hormone-releasing hormone, melanocyte stimulating hormone-release-inhibiting factor (Pro-Leu-Gly-NH_2), and oxytocin. No hydrolysis of thyrotropin-releasing factor or bradykinin potentiating nonapeptide from *B. jararaca* is observed. Relipidated and apoprotein tissue factor act at identical rates under the conditions of the assay. Dansylation and chromatography of tissue factor–peptide incubation mixtures further indicate that relipidated and apoprotein tissue factor also hydrolyze peptides by identical mechanisms. No fewer than six bonds are hydrolyzed in bradykinin while the angiotensins and substance P are degraded to constituent amino acids. Only the N-terminal alanine is released from fibrinopeptide A. 2-Mercaptoethanol greatly inhibits the hydrolysis of bradykinin by relipidated tissue factor.

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