PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **180**, 518–526 (1985)

Thrombin-Elicited Contractile Responses of Aortic Smooth Muscle (42211)

DANIEL A. WALZ, GORDON F. ANDERSON, RAYMOND E. CIAGLOWSKI, MARTHA AIKEN, AND JOHN W. FENTON II*

*Departments of Physiology and Pharmacology, Wayne State University School of Medicine, Detroit, Michigan 48201, and *Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, New York 12201*

Abstract. Human α-thrombin at physiologically relevant concentrations of 0.75 to 225 nM (0.01 to 22 clotting units/ml) caused rabbit thoracic aorta to slowly contract in isolated organ baths. Near maximum contractile tension (10% below the irreversible contraction caused by 22 units/ml) was slowly generated by 75 nM α-thrombin (8 units/ml) over 18 min and was 50% that of the norepinephrine (NE) control. The initial tonus could only be regained by repeated washing and 60 min equilibration. Tissues were refractory to a second 75 nM α-thrombin challenge but responded fully to 1.0 μM NE. Conversion of human α-thrombin to nonclotting but estero/amidolytically active γ-thrombin (<0.1% clotting activity) or nitration of the enzyme (1% clotting activity) did not interfere with the contractile activity, whereas chemical conjugates of the parent enzyme at the catalytic site were inactive. Neither atropine, phentolamine, nor indomethacin blocked the thrombin-induced contractions, whereas D-600 was markedly inhibitory. Preincubation of the tissue with inactive forms of thrombin did not prevent the α-thrombin-induced response. Removal of the vascular endothelium did not prevent contraction. Aorta preparations with intact endothelium relaxed in the presence of very low concentrations (0.75 to 750 pM) of α-thrombin prior to contracting in response to higher concentrations during cumulative dose–response experiments. Our data suggest that catalytically active thrombin forms (α- or nonclotting β- and γ-thrombins) may have hemostatic functions at the vascular levels in hemostasis.

The serine proteinase α-thrombin is generated from its circulating zymogen prothrombin in the final preclotting events of blood coagulation. It is produced in greater concentrations than any of the preceding enzymes of the coagulation pathways and is the central bioregulatory enzyme in hemostasis. Its functions include activation or transformation of plasma proteins (e.g., fibrinogen, factors V, VIII, XIII, and protein C); stimulation of platelets, endothelial cells, and other cell types as well as initiation of mitogenic events in various cultured cells, suggesting its participation in wound healing (1). In addition to its better known enzymic functions, catalytically inactivated forms of α-thrombin have recently been found to possess biological activities with leukocytes, neuroblasts, and other cells (2–4). The enzyme furthermore binds specifically to an endothelial cell protein termed thrombomodulin (5). Such complex formation between α-thrombin and thrombomodulin renders the enzyme inactive with fibrinogen but still permits it to proteolytically activate plasma protein C. Thrombomodulin is not universally present in endothelial cells; it is more prevalent in capillary endothelium and virtually absent in larger vessel endothelium, such as the aorta (5).

Vascular endothelial injury has been experimentally induced directly by α-thrombin or by thrombi (6), and vascular damage has been well recognized with respect to the development of atherosclerotic lesions. Platelets and other blood elements are reported to be spasmogenic with isolated arteries (7) where vasoactive substances in blood may include serotonin, prostaglandin derivatives, as well as thrombin (8–12).

The present studies were undertaken with well-characterized preparations of human α-thrombin and derivative forms in order to assess their ability to induce relaxation or contractile responses in isolated preparations of rabbit thoracic aorta. In contrast to the contractions induced by norepinephrine (NE), only catalytically active thrombin forms induced contractions, which were slow to develop, sustained for prolonged periods of time, and resistant to secondary α-thrombin chal-

0037-9727/85 $1.50

lenge. The concentrations which induced near maximal contractions corresponded to those of α-thrombin generated during blood clotting, although concentrations three orders of magnitude lower evoked a small relaxation response.

Materials and Methods. *Thrombin preparations.* Human α-thrombin was prepared from fraction III and evaluated for enzymic purity by methods previously described (13). Specific activities for the clotting of 2.0 mg/ml fibrinogen solutions were expressed in U.S. "NIH" equivalent units per milligram. Active site titrations were performed with NPGB and were expressed as the percentage of active enzyme with this titrant. Contents of α-, β-, and γ-thrombins were determined by labeling the active-site serine with [^{14}C]diisopropylphosphofluoridate (iPr$_2$P-F), quantitating the isotope distribution after electrophoresis and expressing the enzyme forms as percentages (Table I). Nonclotting γ-thrombin was made by controlled passage at pH 6.2 of α-thrombin through trypsin immobilized on agarose as described (14). Nitrated (NO_2-α-thrombin) and catalytically inactivated forms (iPr$_2$P-, $MeSO_2$-, and D-Phe-Pro-Arg-CH_2-α-thrombins) were made from the parent enzyme form as described elsewhere (15). Throughout, the M_r of 36,600 for the parent enzyme was assumed for the derivative forms, and all thrombin preparations were characterized by the preceding criteria. A specific clotting activity of 3000 units/mg for α-thrombin was assumed in all calculations.

Tissue preparations. Thoracic aorta tissue was obtained from freshly sacrificed 2.0- to 2.8-kg New Zealand White rabbits and immersed in 4°C balanced salt solution (BSS) consisting of 125 mM NaCl, 4.7 mM KCl, 15 mM $NaHCO_3$, 1.2 mM Na_2HPO_4, 12 mM dextrose, 2 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4,

TABLE I. PROPERTIES OF THROMBIN PREPARATIONS AND CONTRACTILE RESPONSES OF AORTIC SMOOTH MUSCLE PREPARATIONS[a]

Preparations and characterization	Modification and description	Norepinephrine response (%)
α-Thrombin (#89) (94.4% α: 2.8 β: 2.9% γ) 91.7% NPGB active 3,400 clotting units/mg	Native enzyme form with high fibrinogen clotting and all other thrombin-ascribed activities.	49.6 ± 1.02 (n = 6)
γ-Thrombin (#31) (0.01% α: 15.7% β: 87.3% γ) 80.0% NPGB active 1.74 clotting units/mg	Thrombin B chain proteolytically cleaved into 3 noncovalently associated fragments. This enzymically active form has essentially no clotting activity.	46.3 ± 7.3 (n = 4)
NO_2-α-Thrombin (#160L) (99.6% α: 0.2% β: 0.2% γ) 57.3% NPGB active 34.3 clotting units/mg	Nitration of ~4 out of 10 tyrosines in α-thrombin with tetranitromethane causes differential loss of clotting versus catalytic activity.	52.4 ± 4.08 (n = 4)
iPr$_2$P-α-Thrombin (#107B) (96.5% α: 2.8% β: 0.7% γ) 0.1% NPGB active 0.5 clotting units/mg	Covalent conjugation of active-site serine (Ser_{195} in chymotrypsin) with iPr$_2$P-F causes enzymic inactivation with a large obstructive conjugated group at the catalytic site.	4.6 ± 0.99 (n = 5)
$MeSO_2$-α-Thrombin (#153) (97.6% α: 0.8% β: 1.6% γ) 0.2% NPGB active 0.3 clotting units/mg	Covalent conjugation of the active-site serine with $MeSO_2F$ causes enzymic inactivation with a small conjugated group at the catalytic site.	3.9 ± 0.86 (n = 5)
D-Phe-Pro-Arg-CH_2-α-Thrombin (#184) (95.7% α: 2.8% β: 1.5 γ) 0% NPGB active 0.1 clotting units/mg	Fibrinopeptide groove obstructed by affinity labeling with D-Phe-Pro-Arg-CH_2-Cl at or near the catalytic site, such that the ligand cannot be displaced, causing essentially complete enzymic inactivation.	0.0 ± 0 (n = 5)

[a] Data assembled from Fenton *et al.* (13, 14) and Sonder and Fenton (15).

aerated with 95% O_2–5% CO_2. Connective tissue was carefully removed and the cleaned artery was slipped over an appropriate size glass rod under BSS for dissection. The artery segment was cut into a spiral helical strip 4 to 5 mm wide at a 45° angle through its entire length. The spiral strip was then bisected lengthwise into two 2- to 2.5-mm-wide strips. Segments ~15 mm, unstretched length, were anchored with 5-0 silk in 10 ml isolated organ baths containing BSS at 37°C. Isometric tension was monitored with a Grass FT-03C force displacement transducer and recorded on a Beckman 411 pen writing recorder. A 60-min equilibration period in the bath was permitted before 4.0 gm of passive tension was placed on each muscle strip. Drugs were added directly to the bathing medium, and all drug or thrombin concentrations are reported as final bath concentrations.

Cumulative dose reponse curves to NE were performed on eight thoracic aorta preparations. The maximal isometric tension was found to occur at 1.0 μM bath concentration and was selected as the standard contractile amplitude to which all subsequent thrombin responses in this paper are compared. After equilibration and passive tension adjustment, 1.0 μM NE additions were repeated, with 10-min washouts and recovery periods, until consistent contractile responses of the same magnitude were obtained. These contractions served as the control 100% NE response for each of the individual tissue observations made and reported herein.

Sources. Human fraction III paste was obtained through the courtesy of Dr. Bryan H. Landis, Armour Pharmaceutical Company, Kankakee, Illinois; D-phenylalanyl-L-propyl-L-arginine chloromethyl ketone (D-Phe-Pro-Arg-CH_2Cl) was a gift from Dr. Elliot Shaw, Brookhaven National Laboratory, Upton, New York. Other drugs and reagents: atropine sulfate, Aldrich Chemical Company, Milwaukee, Wisconsin; phentolamine, Ciba-Geigy Laboratories, Summit, New Jersey; reagent grade salts from Fisher Scientific, Detroit, Michigan; carbachol, indomethacin, norepinephrine tartrate (NE), and trypsin from Sigma Chemical Company, St. Louis, Missouri.

Results. Contractions induced by 75 nM thrombin (8 clotting units/ml) were examined on five stored (overnight in BSS, 4°C) and six fresh aortic muscle preparations (Fig. 1). With all 11 preparations, the enzyme promoted a slow gradual response that became maximal in 17 min 44 sec (±53.5 sec, SEM). With fresh preparations, this response was 49.6%, while stored preparations responded 32.3% that of the NE control. After the sustained contraction induced by 75 nM α-thrombin, repeated washing resulted in a gradual decrease of tonus and required 60 min to reach baseline. When the same (75 nM) α-thrombin concentration was added again to recovered tissues, either no response could be elicited, or a very reduced contraction of <7% was observed. Nevertheless, 1.0 μM NE added to recovered tissue still caused a prompt contraction that showed little

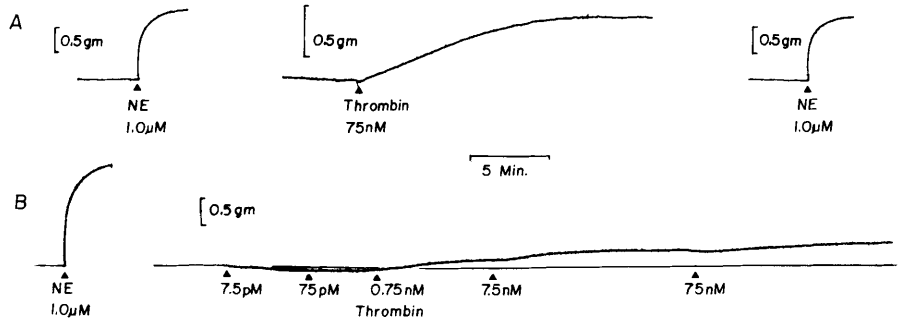

FIG. 1. Responses of rabbit aortic strips to thrombin. (A) Control contractile response to norepinephrine (NE) is shown followed by a single dose of thrombin followed by a 40-min washout period and a second NE response. Note that the thrombin contraction was recorded at a higher sensitivity than norepinephrine controls. (B) Shows a cumulative dose–response to α-thrombin with the baseline superimposed on the recording to show relaxation of the aortic strip to low concentrations of α-thrombin.

or no degradation in amplitude or time course, and in some instances exceeded the 100% NE control. Tissue contraction induced with 180 nM α-thrombin (22 units/ml) generated a maximum sustained contraction which failed to significantly return to baseline after repeated washing (Table II).

To determine if the effect of α-thrombin was mediated through an endothelium released factor, six aortas were carefully dissected to ensure endothelial integrity and in six other preparations the endothelial layer was gently scraped with a scalpel blade. When the maximal NE contraction was initiated in these preparations, 1.0 μM carbachol was added to the organ bath. Those muscles with an intact endothelium relaxed 54%, while the scraped preparations continued their sustained contractures (Fig. 2). All of the tissue preparations, with or without endothelium, contracted when 75 nM α-thrombin was present in the bathing medium. This demonstrated a direct action of the enzyme on the subendothelial tissue and not requiring the intact endothelium.

Cumulative dose–response experiments with α-thrombin were performed on six fresh muscle preparations over a concentration range of 0.75 pM to 75 nM at 10-min intervals. Concentrations of α-thrombin at 7.5 and 75 pM produced gradual relaxation (Fig. 1) of all aortic strips studied but at $\geq$750 pM concentrations a gradual contracture was evident. The maximum contractile response to α-thrombin administered incrementally resulted in a smaller maximum response when compared to the single addition of 75 nM enzyme (Fig. 1).

Selectively modified thrombin preparations were examined to determine whether the contracture response was (i) restricted to α-thrombin like the clotting of fibrinogen; (ii) required a catalytically active thrombin form to carry out an enzymic function; or (iii) involved high-affinity binding in the occupancy of a receptor. In these experiments, the nonclotting forms γ- and NO_2-α-thrombin both possessed intrinisic contractile activities similar to native α-thrombin (Table I and Fig. 3). On the other hand, the catalytically inactivated forms of the enzyme, iPr$_2$P-α-, MeSO$_2$-α-, and D-Phe-Pro-Arg-CH$_2$-α-thrombin, exhibited <5% or no contractile activity. Thus, enzymic activities, but not those necessarily related only to clotting activity, were required for contractile activity.

In each instance where D-Phe-Pro-Arg-CH$_2$-, iPr$_2$P-, or MeSO$_2$-α-thrombin failed to induce contraction, the subsequent addition of 75 nM α-thrombin initiated a normal contraction response demonstrating that the catalytically inactivated thrombin forms could not compete with the native enzyme for receptor occupancy. After prolonged and repeated washing, each test preparation was refractory to a second α-thrombin challenge but still remained fully responsive to NE. Two aortic strip preparations were treated with 1.0 μM trypsin, which produced a gradual contracture similar in onset to that of thrombin; however, only 27% that of the NE control. These preparations were also resistant to a second challenge with trypsin after washout but differed from thrombin in that the subsequent response to NE was sharply reduced,

TABLE II. CONTRACTILE RESPONSE AND RECOVERY TO VARIOUS α-THROMBIN CONCENTRATIONS

Thrombin concentration[a]	% NE response[b]	% Initial tension[c]		
		30	60	90
7.5 nM (0.8 units/ml)	27.4 ± 1.84 (n = 3)	12.7 ± 3.88	4.4 ± 2.61[d]	2.0 ± 0.74
75 nM (8 units/ml)	49.6 ± 1.02 (n = 6)	18.4 ± 3.45	2.7 ± 0.34[d]	2.1 ± 0.81
180 nM (22 units/ml)	53.7 ± 3.08 (n = 3)	87.4 ± 7.07	73.1 ± 6.70[e]	68.7 ± 8.41[e]

Note. See text for experimental details of muscle preparations.

[a] Final bath concentrations.

[b] Percentage of contractile response elicited by 1.0 μM norepinephrine; n = number of tissue samples examined at this thrombin concentration.

[c] Percentage of maximum thrombin contractile response remaining following tissue washing. Each tissue was washed with aerated fresh balanced salt solution at 10-min intervals and tension remaining at 30, 60, and 90 min was recorded.

[d] $P < 0.01$ using Student's t test (unpaired).

[e] Not significantly different.

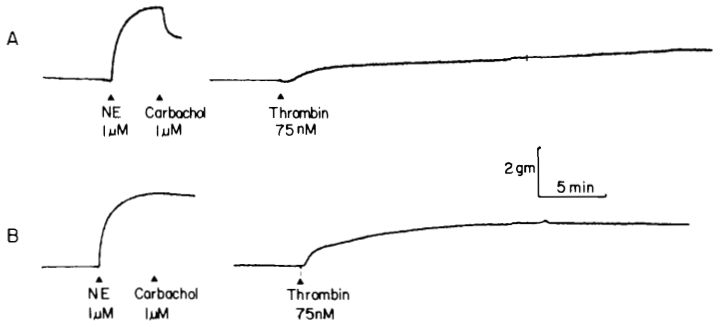

FIG. 2. Response of rabbit aortic muscle strips to α-thrombin. (A) The endothelium was intact as evidenced by the relaxation of the norepinephrine contraction when 10 μM carbachol was present in the bathing medium. (B) The endothelium was removed as described in text and carbachol did not relax the norepinephrine contraction.

suggesting that the NE receptor binding sites had been partially destroyed by proteolytic digestion with trypsin but not with α-thrombin.

High concentrations of selected pharmacologic antagonists were employed to examine the mechanism(s) of the thrombin-initiated contractile response (Table III and Fig. 4). Of the four antagonists examined, atropine modified the contracture least, while phentolamine slightly reduced the response, and D-600 had the greatest blocking activity on the thrombin

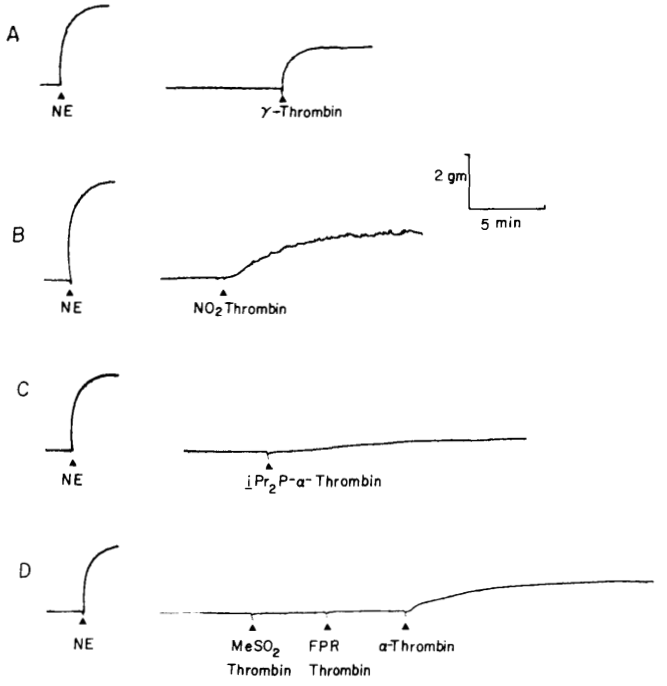

FIG. 3. Response of rabbit aortic smooth muscle strips to thrombin analogs. In each panel the norepinephrine (NE) control response precedes the addition of a thrombin analog. (A) γ-Thrombin, 75 nM, promoted a relatively faster contraction than any other thrombin analog. (B) Nitrothrombin (NO_2 thrombin, 75 nM). (C) Diisopropyl phosphofluoridate-treated thrombin (DFP, 75 nM) i-Pr_2P-α-thrombin. (D) Seventy-five nanomolar each of mesyl thrombin ($MeSO_2$ thrombin), D-Phe-Pro-Arg-CH_2-α-thrombin (FPR thrombin), and 75 nM α-thrombin.

TABLE III. ANTAGONISM OF AORTIC SMOOTH MUSCLE CONTRACTURES BY SELECTED PHARMACOLOGIC INHIBITORS

Antagonist	Incubation lit	Incubation time[b] min	Mechanism of action	% NE response to 75 n*M* α-thrombin[c]
None	—	—	—	49.6 ± 1.02 (n = 6)
Atropine	10	5	Muscarinic inhibitor	45.2 ± 3.2[d] (n = 4) (91.1)
Indomethacin	10	15 to 20	Arachidonate cyclooxygenase inhibitor	31.7 ± 1.6[e] (n = 12) (63.9)
Phentolamine	10	5	α-Adrenergic receptor inhibitor	27.6 ± 4.2[e] (n = 4) (55.5)
D-600	10	5	Ca^{2+} channel blocker	13.8 ± 4.8[e] (n = 4) (27.9)

Note. See text for experimental details regarding muscle preparations.
[a] Final bath concentration.
[b] Amount of preincubation time prior to addition of α-thrombin.
[c] Percentage number in parenthesis is the response relation to α-thrombin control.
[d] Not significantly different.
[e] $P < 0.01$ using Student's t test (unpaired).

contracture. In the 12 indomethacin-treated preparations studied, one tissue was completely blocked by pretreatment, while the remaining preparations exhibited the characteristic slow contracture with minimal depression of the maximum contracture. However, each

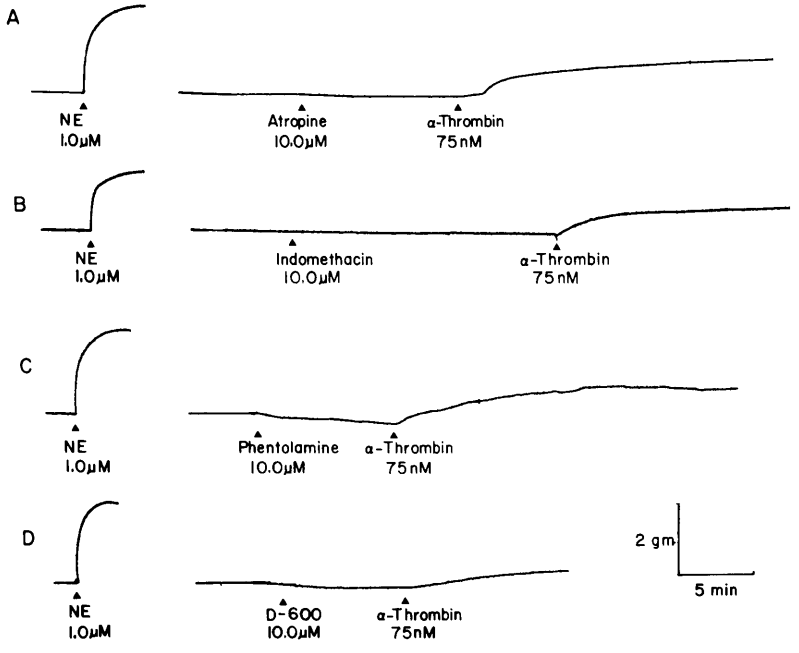

FIG. 4. Response of rabbit aortic muscle strips to α-thrombin in the presence of high concentrations of pharmacologic antagonists; see text and Table III for further description and summary of experiments.

responding preparation reached its maximum contracture amplitude in a shorter period of time. The ability of phentolamine to partially antagonize the thrombin contracture may be offset by the ability of this agent to depress the resting tone of the aortic strips and therefore reduce the magnitude of thrombin contractions to 27.6% of the control NE contraction.

Discussion. Normal human plasma contains sufficient prothrombin to generate ~150 clotting units/ml (14, 16). Assuming a specific clotting activity of 3000 clotting units/mg protein and an M_r of 36,600 for human α-thrombin, this corresponds to 1.37 μM and represents the maximum possible thrombin concentration. However, during the spontaneous clotting of plasma or freshly drawn whole blood, clotting activities do not exceed ~10% of the upper limit, although >90% of the prothrombin is converted to the activation product F 1 • 2 and, presumptively, thrombin (16). Furthermore, clotting activity rapidly rises prior to and peaks with onset of clotting, suggesting that α-thrombin is actively consumed during the clotting process (16, 27). In addition to measuring thrombin generation by the traditional clotting assay, a radioimmunoassay has detected the enzyme initially at 80 pM (3 ng/ml) plateauing at 1.1 to 1.4 nM (40 to 50 ng/ml) concentrations (18). Assuming the peak value of ~15 units/ml (17), the upper α-thrombin concentration generated during clotting is 140 nM.

In the present studies, a threshold concentration as low as 750 pM human α-thrombin was observed to initiate a detectable contractile response with rabbit thoracic aorta preparations in an organ bath at 37°C (Fig. 1), a finding similar to that of Haver and Namm (12). Below this concentration, aorta preparations were found to relax. In this regard, α-thrombin is known to stimulate prostaglandin synthesis in cultured endothelial cells (19), as well as to induce "rounding up" shape changes in cultured cells (20, 21). Since the endothelium should act as a cellular barrier to constituents of or generated in blood (e.g., α-thrombin), and since the contractile responses to higher doses of thrombin were not mediated by the endothelium (Fig. 2), such shape changes may be important in exposing the subendothelium to blood or blood-generated substances.

The sustained contracture produced by 75 nM α-thrombin with rabbit thoracic aorta muscle strips was of relatively low magnitude and slow to develop when compared to NE controls. This contractile action of the coagulation enzyme could be demonstrated with tissue preparations with intact endothelium, as well as endothelial free preparations. Therefore, the response is not mediated by some endothelial originating factor, even though α-thrombin can promote the synthesis of various prostaglandins from vascular endothelial cells (19). Furthermore, a prostaglandin-mediated mechanism is unlikely since indomethacin did not abolish the response even after preincubation with 10 μM indomethacin for 15 to 20 min prior to the addition of α-thrombin. The contracture was furthermore incompletely blocked by 10 μM phentolamine and therefore appears not to be mediated through an α-adrenergic receptor. In this regard, following recovery from a 75 nM α-thrombin response, the tissue remained responsive to NE even though the preparation was refractory to a second addition of α-thrombin, implying that the muscle possessed functional α-adrenergic receptor-mediated mechanisms. The refractoriness to a second α-thrombin challenge could be explained by a very low dissociation and tight binding of thrombin to a receptor site. However, such a high-affinity binding receptor occupancy mechanism should eventually be reversible where α-thrombin could again induce the contracture. If the response is due to an enzymatic cleavage of some active peptide or directly on some receptor mediating a Ca^{2+} influx, it differs from that of trypsin, which also promotes aortic smooth muscle contracture of a low magnitude and slow onset. Exposure to trypsin irreversibly damages the muscle while at the same time sharply reducing the responsiveness to NE, most likely by proteolysis of the NE receptor site as well as other plasma membrane proteins.

Both nonclotting γ- and NO_2-α-thrombin forms were as active as the native α-thrombin form and the catalytically inactivated iPr$_2$P-α-, MeSO$_2$-α-, and D-Phe-Pro-Arg-CH$_2$-α-thrombin forms gave little or no response (Table I), in agreement with one report (12) but not supporting the observation (22) that heated thrombin retained activity. Our findings that washed recovered tissue previously exposed to

75 n*M* α-thrombin was nonresponsive to a second equal dosage of the enzyme also implies a consumptive process. Such a consumptive process, where the active enzyme consumes its receptor, would abate the response or create tolerance to the slow contractile response of α-thrombin.

Incubation of tissues with the catalytically inactivated thrombin forms did not diminish or enhance the subsequent response to thrombin, implying that occupancy of high-affinity receptors for thrombin was not of limiting importance. The finding that γ-thrombin was as active as the native thrombin would favor a low-affinity receptor model, where a catalytically active enzyme carries out a proteolytic function in generating the stimulating signal. In fibroblast culture systems, γ-thrombin, like the native enzyme, stimulates ion fluxes at low-affinity receptors, whereas $i\mathrm{Pr}_2\mathrm{P}$-α-thrombin binds to high-affinity receptors for the native enzyme but does not stimulate ion fluxes (4). Although Haver and Namm (12) observed inhibitory effects in Ca^{2+} free medium, they were unable to demonstrate antagonism with the Ca^{2+} channel antagonists nifedipine and verapamil. Of the potential antagonists we examined, only the Ca^{2+} channel blocker D-600 was a potent inhibitor of the thrombin induced contractile response (Table III and Fig. 4).

In a number of ways our findings are similar to another (12) in that α-thrombin-induced contractions were associated with tolerance. In our studies a second challenge from 75 m*M* α-thrombin, after washout and return to resting tension, resulted in nearly complete loss of contractile activity. Haver and Namm (12) reported that their aortic ring preparations responded to a readdition of thrombin in a manner dependent upon the initial dose and time of exposure; higher initial thrombin concentrations or longer exposure times resulted in a decreased, but never absent, response to thrombin. Both studies show that contracture is refractory to indomethacin. Our results are similar to those which used canine basilar artery to demonstrate the reduction in thrombin contractures by Ca^{2+} channel antagonists (23). The thrombin-induced contracture was both slow to develop and recover where both may also be of hemostatic value. The maximum contracture occurred with 22 units/ml or $\sim$180 n*M* thrombin and was an irreversible contracture, which would not decrease upon repeated washings (Table II). This suggests that "clotting generated contractions" by α-thrombin might be lethal to certain tissues. In contrast, the submaximum contractures induced by 8 units/ml or $\sim$75 n*M* thrombin (a presumptive clotting generated concentration) were slowly reversed over 60 min by repeated washing (Figure 1). In contrast, as reported by others (10, 12, 22) very low α-thrombin concentrations at <0.008 units/ml or 75 p*M* caused weak relaxation of the aortic smooth muscle. This suggests that as α-thrombin is generated it initiates a progression of events, in addition to those better known events of blood clotting and that the slow sustained responses of the blood vessels may be of underlying importance in hemostasis.

Supported by NIH research Grants AG 03539, HL 13160, and HL 27073, and a Grant-in-Aid from the American Heart Association of Michigan.

1. Fenton JW II. Thrombin specificity. Ann NY Acad Sci **370**:468–495, 1981.
2. Bar-Shavit R, Kahn A, Wilner GD, Fenton JW II. Monocyte chemotaxis: Stimulation by specific exosite region of thrombin. Science (Washington DC) **220**: 728–731, 1983.
3. Snider RM, McKinney M, Fenton JW II, Richelson E. Activation of cyclic nucleotide formation in murine neuroblastoma NIE-115 cells by modified thrombins. J Biol Chem **259**:9078–9081, 1984.
4. Stiernberg J, Carney DH, Fenton JW II, La Belle EF. Initiation of DNA synthesis by human thrombin: Relationships between receptor binding, enzymic activity, and stimulation of Rb influx. J Cell Physiol **120**: 289–295, 1984.
5. Esmon NT, Owen WG, Esmon CT. Isolation of a membrane-bound cofactor for thrombin-catalyzed activation of protein C. J Biol Chem **257**:859–864, 1982.
6. Lough J, Moore S. Endothelial injury induced by thrombin or thrombi. Lab Invest **33**:130–135, 1975.
7. DeMey JG, Claeys M, Van Houtte PM. Endothelium-dependent inhibitory effects of acetylcholine, adenosine triphosphate, thrombin and arachidonic acid in the canine femoral artery. J Pharmacol Exp Ther **222**: 166–173, 1982.
8. White RP, Chapleau CE, Dugdale M, Robertson JT. Cerebral arterial contractions induced by human and bovine thrombin. Stroke **11**:363–368, 1981.
9. Fareed J, Messmore HL, Kindel G, Karczman AG, Fenton JW II. Thrombin induced contraction of iso-

lated smooth muscle. Thromb Haemostasis **46**:201, 1981.
10. Ku DD. Coronary vascular reactivity after acute myocardial ischemia. Science (Washington DC) **218**: 576–578, 1982.
11. Haver VM, Namm DH. Generation of a vasoactive substance in human plasma during coagulation. Blood Vessels **20**:92–98, 1983.
12. Haver VM, Namm DH. Characterization of the thrombin-induced contraction of vascular smooth muscle. Blood Vessels **21**:53–63, 1984.
13. Fenton JW II, Fasco MJ, Stackrow AB, Aronson DL, Young AM, Finlayson JS. Human thrombins: Production, evaluation, and properties of α-thrombin. J Biol Chem **252**:3587–3598, 1977.
14. Fenton JW II, Landis BH, Walz DA, Finlayson JS. Human thrombins. In: Lundblad RL, Fenton JF, Mann KG, eds. Chemistry and Biology of Thrombin. Ann Arbor, Mich., Ann Arbor Science, pp43–70, 1977.
15. Sonder SA, Fenton JW II. Proflavin binding within the fibrinopeptide groove adjacent to the catalytic site of human α-thrombin. Biochemistry **23**:1818–1823, 1984.
16. Aronson DL, Stevan L, Ball AP, Franz BB Jr, Finlayson JS. Generation of the combined prothrombin activation peptide (F 1 · 2) during the clotting of blood and plasma. J Clin Invest **60**:1410–1418, 1977.
17. Liu CY, Nossel HL, Kaplan KL. The binding of thrombin to fibrin. J Biol Chem **254**:10420–10425, 1979.
18. Shuman MA, Majerus PW. The measurement of thrombin in clotting blood by radioimmunoassay. J Clin Invest **58**:1249–1258, 1976.
19. Weksler BB, Ley CW, Jaffe EA. Stimulation of endothelial cell prostacyclin production by thrombin, trypsin, and the ionophore A23187. J Clin Invest **62**: 923–930, 1978.
20. Galdal KS, Evensen SA, Brosstad F. Effects of thrombin on the integrity of monolayers of cultured human endothelial cells. Thromb Res **27**:575–584, 1982.
21. Laposata M, Dovnarsky DK, Shin HS. Thrombin-induced gap formation in confluent endothelial cell monolayers in vitro. Blood **62**:549–556, 1983.
22. White RP, Shirasawa Y, Robertson JT. Comparison of responses elicited by alpha-thrombin in isolated canine basilar, coronary, mesenteric, and renal arteries. Blood Vessels **21**:12–22, 1984.
23. White RP, Cunningham MP, Robertson JT. Effect of the calcium antagonist nimodipine on contractile responses of isolated canine basilar arteries induced by serotonin, prostaglandin F_{2a}, thrombin, and whole blood. Neurosurgery **10**:344–348, 1982.

Received April 3, 1985. P.S.E.B.M. 1985, Vol. 180.
Accepted July 24, 1985.