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ACCELERATED COMMUNICATION

Action of Thrombin Receptor Polypeptide in Gastric Smooth Muscle: Identification of a Core Pentapeptide Retaining Full Thrombin-Mimetic Intrinsic Activity

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SUMMARY

We have used a guinea pig gastric longitudinal (LM) smooth muscle bioassay system to evaluate the contractile activities of a previously described thrombin receptor-derived polypeptide, $S_{42}FLLRNPNDKYEPF_{55}$ (one-letter amino acid code) (TRP_{42-55}) and of a series of peptides derived from this sequence. The contractile activities of the polypeptides were compared with the actions of thrombin. Shortened peptides of the sequences $S_{42}FLLRNPND_{50}$, $S_{42}FLLRN_{47}$, and $S_{42}FLLR_{46}$ (TRP_{42-46}) all exhibited contractile activities that were equivalent to or greater than those of the parent polypeptide, TRP_{42-55}. Both TRP_{42-55} and TRP_{42-46} mimicked the action of thrombin, in terms of two different signal transduction pathways that were activated either in the LM preparation or in the related but distinct gastric circular muscle assay. In the LM preparation, the peptide FSLLR also exhibited appreciable, but much reduced, activity. Minimal activity was exhibited in the LM by the sequence SFLLA, but the lysine-containing analogue $S_{42}FLLK_{46}$ was about one fifth as potent as TRP_{42-46}. In contrast, the receptor-derived sequences $S_{42}FLL_{45}$, $S_{42}FL_{44}$-NH_2, $F_{43}LLR_{46}$, and $S_{42}ALLR_{46}$, as well as arginine-containing polypeptides beginning with the SF motif, SFRG and SFRGHITR, were inactive in the LM bioassay system, at concentrations of ≥200 μM, as either agonists or antagonists against TRP_{42-55}. In addition to its actions in the LM and circular muscle preparations, the active pentapeptide, TRP_{42-46}, also exhibited thrombin-mimetic intrinsic activity in a rat aortic arterial ring relaxation bioassay, whereas the pentapeptide $S_{42}FLLA_{46}$ and the tetrapeptide $S_{42}FLL_{45}$ were inactive. We conclude that the intrinsic biological activity of the thrombin receptor-derived peptide resides in the pentapeptide TRP_{42-46} and that the phenylalanine and arginine residues at positions 43 and 46 play key roles in the activity of this pentapeptide in smooth muscle systems.

Quite apart from its widely recognized action as a coagulation factor, thrombin is now known to act on a wide variety of target tissues, so as to exhibit both vasoactive and mitogenic activities (1–8). In vascular preparations, thrombin can cause either an endothelium-dependent relaxation or an endothelium-independent contraction (2–6, 8). Thus, in a sense, the cellular actions of thrombin can be seen in the context of the activities of a number of vasoactive mitogenic polypeptides, such as vasopressin and angiotensin II, and the transmembrane signaling pathways whereby thrombin causes its effects appear to have much in common with the signaling pathways used by a number of peptide hormones (9). Recently, the mechanism whereby thrombin activates its target tissues (e.g., platelets) has been clarified by the elegant work of Coughlin and colleagues (10, 11), who used an expression cloning approach to identify the thrombin receptor, which is structurally related to the guanine nucleotide-binding protein-coupled family of receptors. Upon binding to its receptor, thrombin cleaves the receptor amino-terminal portion at serine residue 42. It was hypothesized that this newly revealed amino-terminal sequence, beginning with serine, could act as a "tethered ligand" to activate the receptor (10). In keeping with this hypothesis, it was found that a synthetic tetradecapeptide, TRP_{42-55}, derived from the proteolytically revealed amino-terminal receptor

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ABBREVIATIONS: TRP_{42-55} (P-14), receptor-derived peptide $S_{42}FLLRNPNDKYEPF_{55}$; B-50/GAP-43 (B-50 P), neurally derived growth-associated protein sequence; CM, gastric circular smooth muscle; FP-5, peptide $F_{42}SLLR_{46}$; LM, gastric longitudinal smooth muscle; TRP_{42-50} (P-9), peptide $S_{42}FLLRNPND_{50}$; TRP_{42-47} (P-6), peptide $S_{42}FLLRN_{47}$; TRP_{42-46} (P-5), peptide $S_{42}FLLR_{46}$; TRP_{42-45} (P-4), peptide $S_{42}FLL_{45}$; TRP_{43-46} (FP-4), peptide $F_{43}LLR_{46}$; P-$3NH_2$, $S_{42}FL_{44}$-NH_2; B-50P4, SFRG; B-50 P, SFRGHITR; FP-4, $F_{43}LLR_{46}$; EGF-URO, epidermal growth factor-urogastrone; Ach, acetylcholine; RA, rat aorta.

sequence possessed intrinsic thrombin-like activity, both in terms of platelet aggregation and in terms of activating receptors in a *Xenopus* oocyte receptor transfection system (10).

In our own work, we have been studying the contractile actions of mitogenic growth factors, such as EGF-URO, in a variety of vascular and nonvascular smooth muscle systems (12, 13). Our focus on the effects of mitogenic/vasoactive agents in these smooth muscle bioassay systems led us, in preliminary work, to evaluate the actions of the thrombin receptor-derived peptide TRP_{42-55} in both arterial and gastric smooth muscle strip assays (14).[1] In summary, in vascular strips, we have found that TRP_{42-55} causes either an endothelium-dependent relaxation or an endothelium-independent contraction (14). Further, we have found that, in LM strips, the receptor-derived peptide causes a concentration-dependent thrombin-mimetic contraction.[1] Concurrent work has documented the contractile action of TRP_{42-55} in a rabbit aortic ring preparation (15). Based on our preliminary data with a number of these smooth muscle response systems, we have come to the conclusion that the LM preparation (13), which retains its sensitivity in response to repeated administrations of thrombin and TRP_{42-55},[1] affords one of the most convenient and reliable assay systems in which to evaluate the biological activity of thrombin receptor peptide analogues. The main question we wished to answer is the following: which portion of the TRP_{42-55} sequence is required for its intrinsic biological activity in smooth muscle preparations? In the work reported in this communication, aimed at answering this question, we have used primarily the LM bioassay to assess the activities of a series of peptides derived from the thrombin receptor tetradecapeptide TRP_{42-55}. The data demonstrate that the intrinsic thrombin-mimetic activity of TRP_{42-55} resides in the first five amino-terminal amino acids.

Materials and Methods

Bioassay procedures. The guinea pig gastric LM and CM preparations were obtained, as described previously (13), from male albino Hartley strain guinea pigs. After sacrifice by cervical dislocation, the animals were dissected to obtain the gastric body, which was freed from mucosal elements and cut either along or at right angles to the LM bundles, to yield LM or CM strips (3 × 10 mm), respectively, for use in the bioassays. Tissue strips were mounted in a plastic organ bath (2–4-ml volume) and were bathed in an aerated (95% O_2/5% CO_2) Krebs-Henseleit buffer of the following composition (in mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgCl_2$, 1.2; $NaHCO_3$, 25; KH_2PO_4, 1.2; and glucose, 10; this maintained the pH at 7.4. In keeping with our previous work (13), routine measurements with the CM preparation were done in the presence of 3 μM indomethacin. Because the CM (but not the LM) preparation desensitizes in response to thrombin, each CM preparation could be exposed to thrombin only a single time. Test peptides were added directly to the organ bath, and concentrations were calculated accordingly. Tension (1 g initially, falling to 0.5 g at equilibrium, in about 1 hr) was monitored isometrically, using Statham or Grass strain transducers. After equilibration, each tissue was exposed three times to a test concentration of carbachol (50 nM; see below), at 25-min intervals. The third contractile response, after which responses remained constant, was taken as a standard, against which the actions of other agonists were compared. For the evaluation of the activity of thrombin in the LM preparation, contractions were monitored at a dosing interval of 90 min (desensitization was avoided by using this interval), followed by washing of the tissue three times at 10 min and 70 min after the addition of enzyme. For the evaluation of the activity of the synthetic peptides, it was possible to use a dosing interval of 25 min, with tissue washes at 10 and 15 min after the addition of peptide to the organ bath. Using this protocol, neither the LM nor the CM preparations were desensitized to the peptides. In order to normalize the data obtained with different preparations, so as to construct concentration-response curves, the contractions caused by thrombin and the receptor peptides were expressed for each preparation as a percentage of the contraction caused by 50 nM carbachol. This concentration of carbachol (EC_{25}) was chosen because it caused a contraction that was in the middle of the range of responses elicited by a variety of other agonists in the LM and CM preparations.

Thoracic RA rings (2 mm × 3 mm) were obtained from male albino Sprague-Dawley animals after sacrifice by cervical dislocation. Rings with an intact endothelial layer were mounted in an organ bath, and tension was monitored in response to added agonists, as described in the preceding paragraph for the LM and CM preparations, using the same aerated buffer. The presence of an intact endothelium was ascertained by monitoring a relaxation response to Ach (1 μM) in a preparation that had been submaximally (EC_{75}) precontracted with noradrenaline (0.1 μM). The activities of thrombin and the receptor-derived peptides were also monitored by measuring a relaxation response, as was done for Ach, in a noradrenaline-precontracted preparation; ascorbic acid (0.1 mM) was added to the organ bath to minimize oxidation of the catecholamine.

Peptides and other reagents. All peptides were prepared by standard solid-phase synthesis methods, either in our own laboratory (16) or in the Core Peptide Laboratory at the Department of Biochemistry, Queen's University (Kingston, Ontario, Canada). The compositions of the following synthetic peptides, which were purified to homogeneity (single peak on high performance liquid chromatography), were documented by amino acid compositional analysis: TRP_{42-55}, TPR_{42-50}, TRP_{42-47}, TRP_{42-46}, FP-5, $S_{42}ALLR_{46}$, $S_{42}FLLK_{46}$, $S_{42}FLLA_{46}$, TRP_{42-45}, FP-4, P-3NH_2, B-50P4, and B-50 P. The control basic/hydrophillic peptide B-50 P, derived from the sequence of the B-50/GAP-43 protein (17), was selected because of its amino-terminal sequence homology with the thrombin receptor-derived peptides. The largest receptor-derived synthetic peptide, TRP_{42-55}, was also subjected to mass spectral analysis and was found to have the expected molecular mass. In a human platelet aggregation assay, TRP_{42-55} exhibited an EC_{50} of 20 μM (39 μg/ml), in excellent accord with the published data of Vu *et al.* (10). Weighed peptide samples were dissolved in distilled water or in 50 mM phosphate buffer, pH 7.4, to yield stock solutions, which were added directly to the organ bath; final concentrations were calculated accordingly. Peptide concentrations were verified by quantitative amino acid analysis of stock solution aliquots. Ach, carbachol, and noradrenaline were from Sigma (St. Louis, MO), as was highly purified human thrombin (4000 units/mg; catalogue no. T3010); 1 clotting unit/ml thrombin is approximately equivalent to 10 nM pure enzyme. Tyrphostin (RG50864; also designated AG213) was kindly provided by Dr. R. R. Swillo, Rhône-Poulenc Rorer (Collegeville, PA). Genistein was from ICN Biochemicals (Costa Mesa, CA).

Results

The LM preparation, which was found previously[1] to be the most convenient assay for routine use in evaluating the actions of thrombin and its receptor-derived peptides in a smooth muscle system, was used to screen the contractile activities of all synthetic peptides, to compare the contractile actions of the peptides with the tissue response to thrombin (Fig. 1), and to compare the concentration-effect curves for peptides P-14, P-9, and P-5 (Fig. 2). All of the receptor-derived peptide sequences beginning with serine 42 and possessing five or more residues

[1] S.-G. Yang, A. A. Laniyonu, M. Saifeddine, G. J. Moore, and M. D. Hollenberg. Actions of thrombin and thrombin receptor peptide analogues in gastric and aortic smooth muscle: development of bioassays for structure-activity studies. Submitted for publication.

(i.e., P-14, P-9, P-6, and P-5) were found to exhibit thrombin-mimetic activity in the LM bioassay (Fig. 1 and data not shown). The pentapeptide FP-5, with the first two amino acids reversed, also exhibited low but appreciable activity in the LM assay (Figs. 1 and 2). The pentapeptide with the phenylalanine replaced with alanine ($S_{42}ALLR_{46}$) (≥200 μM), was inactive in the LM assay, either as an agonist or an antagonist; the analogue with arginine replaced by alanine ($S_{42}FLLA_{46}$) (≥400 μM) exhibited minimal contractile activity but failed to act as an antagonist of P-14 (Fig. 2 and data not shown). Preliminary work with a P-5 analogue in which arginine was replaced with a lysine demonstrated that the pentapeptide $S_{42}FLLK_{46}$ exhibited considerable contractile activity, with a potency about one fourth to one fifth that of P-5 (data not shown). The shorter peptides (P-4, FP-4, and P-3NH_2) were inactive at concentrations of ≥400 μM, either as contractile agonists or as antagonists of the actions of the complete receptor-derived peptide (TRP_{42-55}) (data not shown). Similarly, the B-50 protein-derived sequences B-50P4 and B-50 P, basic peptides having the same two amino-terminal amino acids as the receptor-derived sequence, were inactive either as agonists (at ≥200 μM) or as antagonists in the LM bioassay (Fig. 2 and data not shown).

The thrombin-mimetic activities of the receptor-derived pentapeptide P-5, as well as peptides P-9 and P-14, were observed not only in the LM preparation but also in the closely related but distinct CM preparation and in the unrelated endothelium-dependent RA vascular relaxation assay (Fig. 1). In the RA assay, $S_{42}FLLA_{46}$ and P-4 were inactive at concentrations of ≥200 μM (Fig. 1 and data not shown). However, the amino-terminally altered receptor-derived pentapeptide FP-5 exhibited low but appreciable intrinsic thrombin-mimetic activity in the RA preparation, in keeping with the results with the LM assay (Fig. 1). It should be pointed out that, in the RA assay, although FP-5 caused relaxation, the pattern of response differed slightly from the one caused by P-5 (Fig. 1).

The concentration-effect curves for the pentapeptide P-5, the nonapeptide P-9, and the tetradecapeptide P-14 revealed that, in the LM tissue, all of the peptides exhibited comparable but distinct potencies, with EC_{50} values in the range between 2 and 200 μM (Fig. 2). However, we were not able to establish a reliable maximum response to the P-5 pentapeptide in our LM assay system at concentrations as high as 300 μM. Other studies have also encountered difficulties in achieving a maximum response to a receptor-derived peptide (15, 18). Moreover, we were unable, even with the use of protease inhibitors (data not shown), to determine whether peptide proteolysis (albeit unlikely) during the course of the bioassay response (3–5 min) might have affected the measured potencies. Thus, the relative potencies of the peptides, as measured, must be interpreted with caution. Nonetheless, the pharmacological efficacies of P-9 and P-5 appeared to be greater than that of P-14, so as to produce a greater maximum response (Fig. 2). With the reservations outlined above, the relative potencies of the three peptides were P9 > P14 > P5, with estimated EC_{50} values of 4, 14, and 120 μM, respectively. The EC_{50} for P-5 was estimated with the use of a plot of percentage of contraction/[P-5] versus [P-5]; the linear plot yields the EC_{50} as the abscissa intercept.

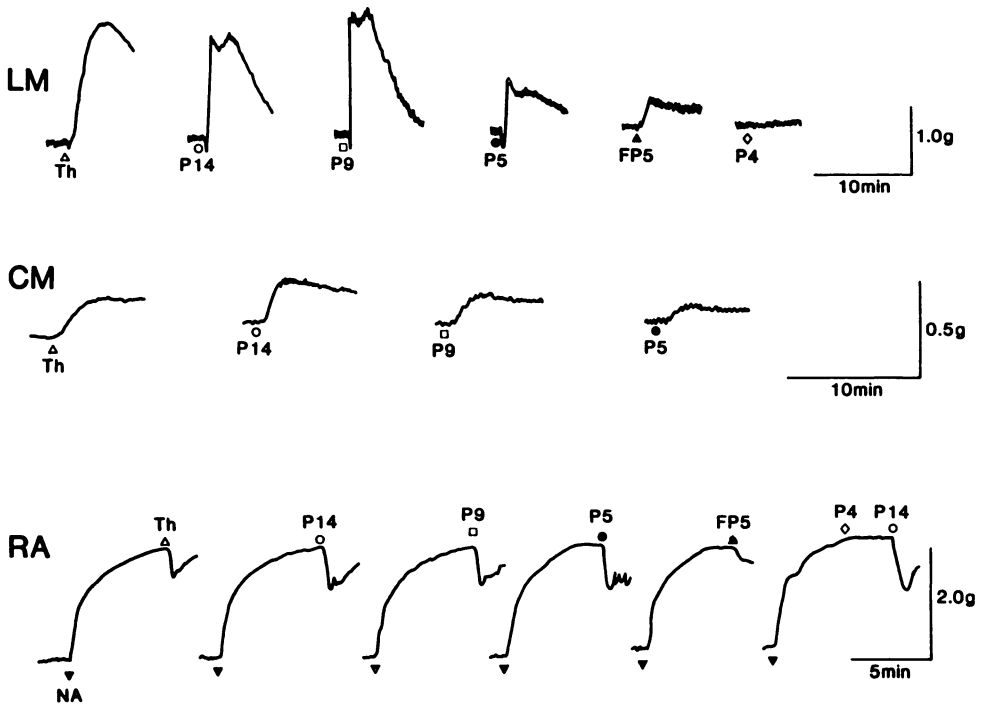

Fig. 1. Effects of thrombin (*Th*) and receptor-derived peptides in guinea pig gastric (LM and CM) and RA assays. Tissue strips were exposed to thrombin (1 unit/ml) or to the receptor-derived peptides P-14 (○), P-9 (□), P-5 (●), FP-5 (▲), and P-4 (◇); tension was recorded isometrically. The increased tension (upward deflection) that developed in the LM (*upper tracings*) and CM (*middle tracings*) preparations was measured directly; the relaxation (downward deflection) of the RA rings (*lower tracings*) was monitored after precontraction of the tissue with 0.1 μM noradrenaline (*NA*) (▼). Each set of tracings shows the sequential responses of single tissue preparations, which were representative of four or more independently conducted experiments. The concentrations of peptides were selected to produce an optimal typical response in each tissue preparation; for the LM prepartions, peptides P-14, P-9, and P-5 were used at 60 μg/ml, whereas peptides FP-5 and P-4 were at 200 μg/ml; for the CM preparations, P-14 and P-9 were at 120 μg/ml and P-5 was at 180 μg/ml; and, for the RA assay, P-14, P-9, and P-5 were at 60 μg/ml, whereas FP-5 and P-4 were at 120 μg/ml. The scales for time and tension for each assay are shown on the *right*. Tissue strips were washed and equilibrated between each exposure to the agonists, as outlined in Materials and Methods.

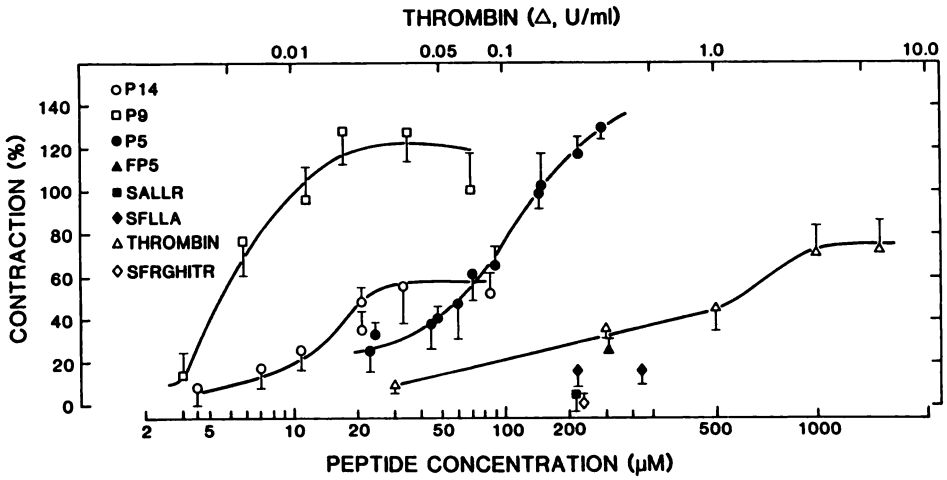

Fig. 2. Concentration-response curves for thrombin and receptor-derived peptides in the LM bioassay. LM strips were exposed to increasing concentrations of either thrombin (△) (*top abscissa*) or receptor-derived peptides (*bottom abscissa*) (●, P-5; □, P-9; ○, P-14). The contractile responses were quantitated in individual tissue strips as a percentage of tension developed in the same tissue strip in response to 50 nM carbachol. The concentration-response curves for each agonist represent data from assays done with tissue from two or more animals, using three or more independent tissue strips in each assay. The figure shows the average response at each concentration, with the standard error represented at each *point* with a *bar*. The tension monitored in response to FP-5 (▲) and in the presence of peptides with little or no activity is also shown (■, SALLR; ◆, SFLLA; ◇, B-50 P). The scale for peptide concentration (μM) is at the *bottom* and that for thrombin concentration (unit/mL) is at the top; 1 clotting unit/ml thrombin is approximately equal to 10 nM pure enzyme.

In our studies with the mitogenic/vasoactive polypeptide EGF-URO, we observed that the contractile responses of the LM and CM preparations to this agent are due to the activation of quite distinct signal transduction pathways, both of which are sensitive to the tyrosine kinase inhibitors tyrphostin (RG50864) and genistein, but only one of which (LM preparation) is mediated via the production of prostanoid metabolites, so as to be blocked by indomethacin (13, 19, 20). We wondered whether thrombin, like EGF-URO, might activate such distinct signal transduction pathways in the LM and CM preparations and, if so, whether the receptor-derived peptides, particularly P-5, might also mimic the action of thrombin in this regard. As illustrated in Fig. 3, *tracing 1A*, the contractile action of thrombin in the LM, but not the CM, preparation was abrogated by indomethacin. Further, in both the LM and CM preparations the tyrosine kinase inhibitor genistein, at a concentration (≤15 μM) known to block selectively the contractile actions of EGF-URO but not to affect the actions of agonists such as carbachol or bradykinin (20), also blocked the contractile actions of thrombin (Fig. 3, *tracing 2A*). Comparable effects were observed with 20 μM tyrphostin (data not shown). In all respects, the effects of indomethacin, genistein, and tyrphostin on the actions of the receptor-derived pentapeptide P-5, as well as the originally described tetradecapeptide P-14, paralleled the inhibition by these agents of thrombin action (Fig. 3, *tracings 1B* and *2B*, and data not shown). That is, indomethacin inhibited the action of the receptor-derived peptides and thrombin in the LM, but not the CM, preparation, and the tyrosine kinase inhibitors blocked the contractile actions of thrombin and the receptor-derived peptides in both the LM and CM preparations, without affecting the contractile actions of agents such as carbachol and bradykinin (Ref. 20 and data not shown).

Discussion

In their studies of the structure-activity requirements of the thrombin receptor for its sensitivity to thrombin activation, Coughlin and colleagues (21) observed that a mutated receptor, lacking the hirudin-like sequence ($K_{51}YEPF_{55}$) in its amino-terminal portion, was insensitive to the action of thrombin in the oocyte transfection bioassay but responded to the receptor-derived peptide TRP_{42-55}. Additionally, work from the Coughlin laboratory (10) indicated that the amino-terminally altered receptor peptide FSLLRNPNDKYEPF was not active either in a platelet aggregation assay or in the receptor transfection oocyte bioassay. Taken together, the data of Coughlin and co-workers thus pointed to the importance of both the amino-terminal serine of TRP_{42-55} and the carboxyl-terminal pentapeptide $K_{51}YEPF_{55}$ for the biological activity of the receptor-derived peptide. We also noted that the carboxyl-terminal portion of TRP_{42-55} ($K_{51}YEPF$) is related to the carboxyl-terminal pentapeptide of the vasoactive/mitogenic peptide angiotensin II (YIHPF), wherein the tyrosine residue plays a key role in receptor activation (22). Therefore, before beginning our study of the receptor-derived peptides, we fully expected a contribution from both the amino-terminal and the carboxyl-terminal portions of TRP_{42-55}.

In contrast to our expectations, summarized in the preceding paragraph, the main finding of our study was that, in the LM and RA bioassays, the intrinsic thrombin-mimetic activity of the receptor-derived amino acid sequence resides in the core amino-terminal P-5 pentapeptide. This pentapeptide motif, SFXLR, is to be found in the human (10), mouse (21), and hamster (23) thrombin receptor sequences. The thrombin-mimetic actions of the receptor-derived pentapeptide were evident not only in two quite different smooth muscle response systems (gastric contraction versus vascular relaxation) but also in the activation of two distinct signal transduction pathways within the same tissue strip (i.e., the indomethacin-sensitive, tyrosine kinase inhibitor-sensitive, LM contractile pathway versus the indomethacin-resistant, tyrosine kinase inhibitor-sensitive, CM contractile pathway). In this regard, the thrombin-triggered signal transduction pathways can be seen to have much in common with those activated by EGF-URO (19, 20). From a structural point of view, the Ser_{42}-containing pentapeptide,

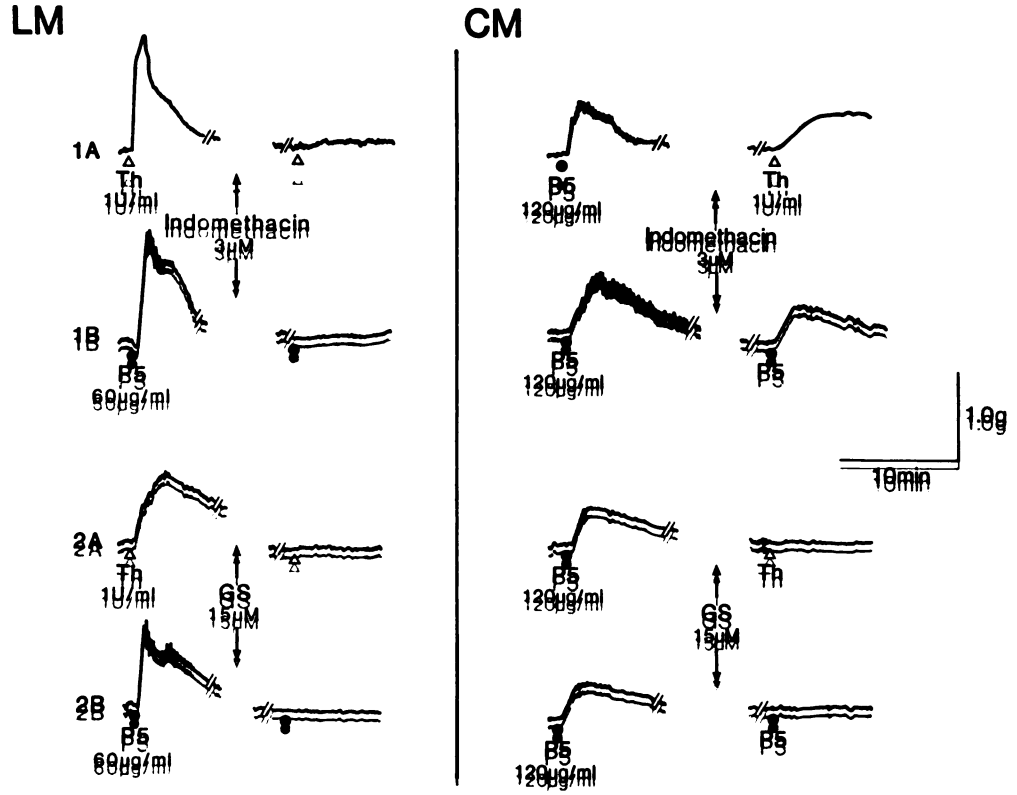

Fig. 3. Effects of indomethacin and genistein on the action of thrombin and receptor-derived peptide P-5 in guinea pig LM and CM strips. Contractile responses to either thrombin (*Th*) (1 unit/ml) (*tracings 1A* and *2A*) or peptide P-5 (*tracings 1B* and *2B*) were measured in LM (*left*) or CM (*right*) strips, either before or after treatment for 20 min with indomethacin (3 μM) (*tracings 1A* and *1B*) or with genistein (*GS*) (15 μM) (*tracings 2A* and *2B*). Because of the thrombin-induced desensitization of the CM preparation, the initial tissue sensitivity of the CM was evaluated by exposure to P-5 (120 μg/ml) before evaluation of the effect of indomethacin or genistein on thrombin action. Each *tracing* illustrates typical responses of either the LM or CM preparations exposed sequentially to the various agonists and inhibitors, as indicated. Tissues were washed and reequilibrated between each addition of agonist, as outlined in Materials and Methods. The data are representative of three or more independently conducted experiments on tissues derived from different animals. The scale for tension and time for all assays is shown on the *right*.

exposed by the action of thrombin on its receptor, yielding a tethered ligand, would be prominently displayed because of the turn caused by the proline residue at position 48. The position of the proline could facilitate the interaction of the tethered pentapeptide ligand with the receptor.

The data obtained for one of the receptor-derived peptides with our smooth muscle bioassays appeared to differ from some of the information reported by the Coughlin group, using either the platelet aggregation assay or the oocyte receptor transfection assay, in that the pentapeptide with an amino-terminal phenylalanine (FP-5) exhibited appreciable, albeit low, intrinsic thrombin-mimetic activity in our assay systems; as mentioned above, the receptor-derived tetradecapeptide with the first two amino-terminal amino acid reversed (i.e., F_{42}SLLRNPNDKYEPF$_{55}$) was reported to be inactive (10). Unfortunately, we have not yet been able to evaluate critically, in a platelet aggregation assay, either the core pentapeptide (TRP_{42-46}) or the pentapeptide beginning with an amino-terminal phenylalanine, to determine whether these analogues might distinguish the platelet receptor from the one present in smooth muscle (see below). Nonetheless, the EC_{50} we have measured for TRP_{42-55} (Fig. 2) in the LM assay (14 μM) is in good accord with the EC_{50} for this peptide either measured by us in a platelet assay ($EC_{50} \cong 20$ μM) or reported by the Coughlin group (10). Unfortunately, the relatively high EC_{50} of P-14 suggests a peptide receptor affinity in the micromolar range, so as to render problematic measurements of receptor binding using a ^{125}I-labeled P-14 probe.

Our data also differ in a number of respects from information that appeared upon completion of our study, documenting the ability of a number of thrombin receptor-derived peptides to activate human platelets and to stimulate hamster CCL39 fibroblast and human megakaryoblastic HEL cell lines (24, 25). For instance, in the platelet assay, P-4 was reported to be active, with an EC_{50} of about 190 μM, whereas this peptide failed to elicit a response at comparable concentrations in either of our assays. Further, in the platelet assay, a hexapeptide with an R_{46} to A_{46} substitution was reported to be active ($EC_{50} \equiv 65$ μM) (22), whereas the analogous peptide studied by us (S_{42}FLLA_{46}) was inactive in the RA bioassay at comparable concentrations. Finally, our data demonstrated that TRP_{42-46} exhibits full intrinsic thrombin-mimetic activity. In this regard, the pharmacological efficacies (as defined classically by Stephenson *et al.* or by the intrinsic activity term of Ariens) of the receptor-derived peptides in the LM assay appear to differ. No comparable measurement seems feasible in the platelet assay. In the reported platelet assays (24), only the hexapeptide TRP_{42-47} was described as exhibiting a high degree of activity ($EC_{50} \equiv 0.8$ μM), whereas information about the pentapeptide TRP_{42-46} was significantly absent from the recorded data, despite a description of studies with a relatively inactive tetrapeptide. Thus, the intrinsic activity (or efficacy) of the penta-

peptide TRP_{42-46} in the platelet system remains an open question, which is presently under study in our laboratory. Further, in the CCL39 fibroblast mitogenesis assay, short receptor-derived peptides did not behave as full agonists but required the concurrent presence of fibroblast growth factor to stimulate thymidine incorporation (25). Possibly, the differences between our own data and the information reported elsewhere (10, 24, 25), in terms of receptor peptide structure-activity relationships, may be due to species differences between the guinea pig, hamster, rat, and human receptors. However, taken together, our own data, along with concurrently published information suggesting significant differences in the structure-activity relationships for the smooth muscle assays on the one hand (FP-5, active; SFLLA and P-4, inactive) and the platelet assay on the other (P-4 and SFLLAN, active; FSLLRNPNDKYEPF, inactive), point to the existence of distinct thrombin receptor subtypes in platelets, smooth muscle (LM and CM), and endothelial cells (RA assay). The work we report here, establishing the key importance of the core amino-terminal serine-containing pentapeptide motif, in terms of the thrombin-mimetic action of the receptor-derived sequence in smooth muscle response systems, provides a basis for exploring further the hypothesis that thrombin receptor subtypes may exist in different tissues.

Acknowledgments

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