ELSEVIER

European Journal of Pharmacology 349 (1998) 237–243

ejp

Disparate effects of thrombin receptor activating peptide on platelets and peripheral vasculature in rats

Madhu S. Chintala *, Peter J.S. Chiu, Vernon Bernadino, Glen G. Tetzloff, Richard Tedesco, Crawford Sabin, Robert W. Watkins, Edmund J. Sybertz

CNS / CV Biological Research, Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033-0530, USA

Received 27 February 1998; accepted 6 March 1998

Abstract

The hemodynamic and platelet effects of the thrombin receptor activating peptide SFLLRN (TRAP) were evaluated in rats. TRAP failed to aggregate rat platelets in vitro (platelet rich plasma) or in vivo in the pulmonary microcirculation. In contrast, TRAP aggregated washed human platelets. Intravenous injection of TRAP (1 mg/kg) in inactin-anesthetized rats produced a biphasic response in blood pressure characterized by an initial depressor response (-25 ± 3 mmHg for 15–30 s) followed by a pronounced pressor response (50 ± 7 mmHg for 2–3 min). This increase in blood pressure can be attributed to increases in total peripheral resistance since cardiac output remained unchanged. Further, only the pressor responses were observed in pithed rats suggesting a direct effect of TRAP in causing smooth muscle contraction. Consequently, rat platelets differ from human platelets in that they are resistant to TRAP whereas rat vasculature is highly sensitive to TRAP. These observations suggest that while the thrombin receptors on rat vasculature may be similar to those on human platelets, the receptors and/or the coupling mechanisms in rat platelets appear different from human platelets.

Keywords: Platelet aggregation; Vascular response; Blood pressure; Pulmonary microcirculation; ^{111}Indium; Labeled platelet; Hemodynamics

1. Introduction

In addition to its role in the coagulation pathway, the serine protease alpha thrombin elicits a variety of cellular responses presumably mediated through activation of specific thrombin receptors. Thrombin is one of the most potent stimulus for platelet aggregation (Fenton, 1988). It also causes the release of endothelial derived relaxing factor (EDRF), prostacyclin and von Willebrand factor from endothelial cells, proliferation of fibroblasts and migration of smooth muscle cells (Awbrey et al., 1979; Bar-Shavit et al., 1990; Chen and Buchanan, 1975; Glusa, 1992). The thrombin receptors from human platelets have been sequenced and cloned (Vu et al., 1991). Thrombin binds to its receptor via its anion binding exosite and generates a new amino terminus by cleaving the receptor after the amino acid residue Arg^{41}. The newly generated N-terminal segment functions as a 'tethered ligand' and activates the receptor. A 14-amino acid peptide SFLLRN-PDNKYEPF which mimics the unmasked new amino terminal is a full agonist for the thrombin receptor activation in human platelets.

Thrombin receptors belong to the seven-transmembrane-spanning domain receptor family coupled to G-proteins and can be desensitized (Dohlman et al., 1991; Gilman, 1987; Vu et al., 1991). Several synthetic peptides corresponding to the wild-type sequence of the human platelet thrombin receptor have been reported to mimic thrombin in eliciting various thrombin-signaled cell responses in different cell types suggesting that these peptides possess intrinsic thrombin-like activity (Huang et al., 1991; Vassalo et al., 1991; Vu et al., 1991). Several investigators have reported that these peptides can cause contraction or relaxation of blood vessels depending on the loci of the blood vessels and species (Gebremedhin et al., 1986; Ku and Zaleski, 1993; Lum et al., 1994; Rapoport et al., 1984; Tesfamariam et al., 1993). In addition species variability in platelet responsiveness to thrombin receptor activating peptides (TRAPs) have been reported in vitro

* Corresponding author. Tel.: +1-908-298-3237; fax: +1-908-298-3294.

0014-2999/98/$19.00

systems (Connolly et al., 1994; Cook et al., 1993; Kinlough-Rathbone et al., 1993).

Several investigators have reported that TRAPs do not activate rat platelets (Connolly et al., 1994; Kinlough-Rathbone et al., 1993). However, the thrombin receptor in the rat vascular smooth muscle has been cloned and shown to have significant sequence homology to the human platelet and hamster fibroblast thrombin receptor (Zhong et al., 1992). Tissue distribution studies revealed the presence of the mRNA for the rat aortic smooth muscle thrombin receptor in the rat kidney, lung and testis. Hence, in the present study we investigated the vascular and hemodynamic effects of TRAP in inactin-anesthetized rats. In addition we used the rat intrapulmonary platelet aggregation model (Chiu and Tetzloff, 1994) to determine if rat platelets would be sensitive to TRAP under in vivo conditions since all previous reports dealing with rat platelets and TRAP were done in vitro.

2. Material and methods

Animal experimentation in this study was carried out in accordance with the National Institutes of Health Guide to the Care and Use of Laboratory Animals and the Animal Welfare Act in a program accredited by the American Association for Accreditation of Laboratory Animal Care.

2.1. In vitro platelet aggregation in rat PRP

Male Charles River CD rats (250–300 g) were anesthetized with inactin (100 mg/kg, i.p.). After an abdominal incision, the aorta was exposed and entered just anterior to the bifurcation with a 21G Vacutainer multiple-sample needle. Donor blood (9 ml) was collected in two citrate Vacutainer tubes (containing 0.5 ml of 3.2% buffered sodium citrate solution). After centrifugation ($130 \times g$ for 15 min), platelet rich plasma was removed and used for the aggregation assay as described in Section 2.1.1. In those studies where amastatin was used the platelets were incubated with amastatin for 2 min before challenge with the agonist. To determine if pretreatment with TRAP causes desensitization of thrombin induced aggregation, rat platelet rich plasma was incubated with 100 μM TRAP for 5 min at 37°C before challenge with 0.1 U/ml of thrombin.

2.1.1. Aggregation assay

Platelet aggregation was performed in a dual channel Chronolog aggregometer (Model 440, Chronolog Corp). Briefly, 0.48 ml of platelet rich plasma was added to the cuvettes and incubated at 37°C for 5 min. Aggregation was initiated by addition of human TRAP or rat peptide (SFFLRN) or thrombin to the platelets and the aggregation response was then monitored for 5 min on an IBM computer and the peak aggregation response was determined turbidimetrically with the help of the Aggro/LINK software (Chronolog).

2.2. In vivo platelet aggregation in the rat pulmonary microcirculation

2.2.1. Preparation of indium labeled platelets

Blood from donor rats were collected as described above. The blood was then centrifuged ($130 \times g$ for 15 min) and the platelet rich plasma was removed and made up to 20 ml with calcium-free Tyrodes solution (CFTS) containing prostaglandin (PGE_1, 0.3 μg/ml). CFTS was prepared by combining solution 1 (sodium chloride 136.9 mM, potassium chloride 2.7 mM, sodium phosphate monobasic 0.4 mM, dextrose 5.6 mM, sodium bicarbonate 11.9 mM) and solution 2 (trisodium citrate 74.8 mM, citric acid 38.1 mM) in a ratio of 10: 1 and adding PEG_1, 0.3 μl/ml of 1 mg/ml stock solution. The diluted PRP was spun at $750 \times g$ for 7.5 min. The pellet was resuspended with 20 ml of CFTS and recentrifuged at $750 \times g$ for 7.5 min. The pellet was then resuspended in 3 ml of CFTS warmed to 37°C for 3 min. Twenty μCi of ^{111}In-oxine was added and incubated for another 3 min. The labeled suspension was pelleted and washed twice (20 ml of CFTS at $750 \times g$ for 7.5 min) and resuspended in 2.2 ml of 0.9% saline.

2.2.2. Monitoring of intrapulmonary accumulation of ^{111}indium-labeled platelets

Rats, fasted overnight were anesthetized with inactin sodium (100 mg/kg, i.p.); the trachea was cannulated (PE-240) to facilitate spontaneous respiration and the left jugular vein was cannulated (PE-50) for injection of platelets and drugs and the animals were placed in a supine position. Rectal temperature was maintained at 37°C with a Yellow Springs thermostat-controlled heating pad. Intravenous infusion of saline at 2 ml/h was initiated. Washed ^{111}indium-labeled platelet suspension (2 μCi in 0.5 ml saline) was injected via the jugular vein. A sodium iodide crystal scintillation detector was placed immediately above the thorax. A quad scintillation pre-amplifier/amplifier and a multiplex/router (Nucleus, Oak Ridge, TN, USA) were used to amplify and select signals from each detector, respectively. Pulses were logged by a processor within a Dell computer, and count rates were displayed graphically (Collimation, Fairfield, NJ, USA).

Aggregatory responses to agonist were determined by summating successive 30 s counts starting 2 min before the challenge and continuing for an additional 10 min after the challenge. All injections were completed within 10 s. Data were transformed to percent change from pre-challenge baseline counts. 'Area under the curve (AUC)' of the '% change vs. time' curve was estimated by a linear trapezoidal method to obtain aggregatory responses to individual agonists.

2.3. In vivo blood pressure responses in rats

Rats were anesthetized and prepared for intravenous injections as described above. In addition the left carotid artery was cannulated (PE-50) and blood pressure was recorded with a Statham pressure transducer connected to a Grass polygraph. In the nephrectomized rats a lateral midline incision was made, the renal arteries isolated and a silk suture was passed around the vessel to facilitate ligature. After a 30 min equilibration period one of the following experiments were carried out.

2.3.1. Effects of TRAP on blood pressure

Vehicle (0.1 ml saline) or TRAP (1 mg/kg, i.v. bolus) were administered i.v. bolus and the changes in blood pressure were monitored for 30 min.

2.3.2. Effect of N^G-nitro L-arginine methyl ester (L-NAME) on the blood pressure response to TRAP

A control blood pressure response to TRAP (1 mg/kg, i.v.) was obtained. When the blood pressure returned to baseline intravenous infusion of L-NAME (0.3 mg/kg per min × 30 min, in saline) was initiated. Twenty five minutes into the infusion the rats were challenged again with TRAP (1 mg/kg, i.v.) and the changes in blood pressure were recorded.

2.3.3. Effect of indomethacin on the blood pressure response to TRAP

A control response to TRAP (1 mg/kg, i.v.) was obtained. Indomethacin (3 mg/kg, i.v., bolus) was then administered and 10 min later a second response to the same dose of TRAP was obtained.

2.3.4. Effects of TRAP in nephrectomized rats

After obtaining a control response to TRAP the renal arteries were ligated with a silk suture and 10 min later a second response to TRAP was obtained.

2.3.5. Effects of TRAP on blood pressure in pithed rats

In the pithed rats studies a control response to TRAP (1 mg/kg, i.v.) was obtained. When the blood pressure returned to baseline the rats were pithed through the right orbit with a steel rod as described by Grant and McGrath (1988) and immediately placed on an artificial respirator. Thirty minutes after pithing, a second response to TRAP was obtained.

2.3.6. Effect of thrombin on the blood pressure response to TRAP

A control response to TRAP (1 mg/kg, i.v.) was obtained. Rat thrombin (50 U/kg, i.v., bolus) was then administered and 10 min later a second response to the same dose of TRAP was obtained

2.4. Hemodynamic studies in rats

Rats were anesthetized and prepared as described above. In addition the caudal artery was cannulated (PE-50) to measure blood pressure using of a Statham pressure transducer connected to a Hewlett Packard chart recorder. The right jugular vein was cannulated with a PE-50 catheter and advanced into the right auricle for injection of indicator for measurement of cardiac output via thermal dilution as described by us previously (Baum et al., 1986).

2.5. Drugs and chemicals

SFLLRN (TRAP) was purchased from Bachem Bioscience (Philadelphia, PA), human thrombin was purchased from Enzyme Research (South Bend, IN), L-NAME, indomethacin and all other chemicals were purchased from Sigma Chemical (St. Louis, MO). The rat peptide SFFLRN was synthetized in-house by the chemists at Schering-Plough research institute.

2.6. Statistical analysis

All data are expressed as mean ± S.E.M. Statistical evaluation of the data was made using repeated measures of analysis of variance and Scheffe *F*-test. Values with $P < 0.05$ were regarded as significant.

3. Results

3.1. Platelet aggregation in vitro

TRAP caused a concentration-dependent aggregation of washed human platelets with an IC_{50} of 8.5 μM (data not

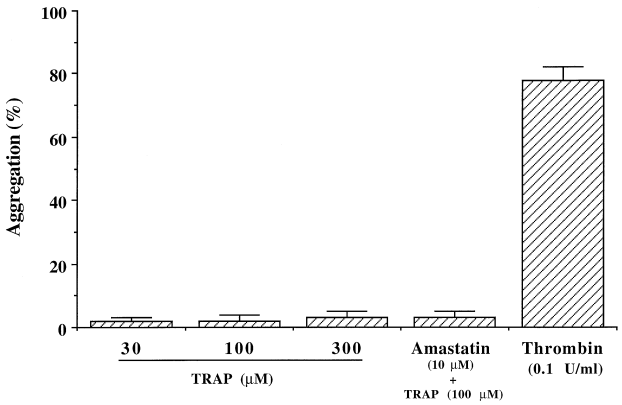

Fig. 1. Aggregatory responses of rat platelet rich plasma to TRAP alone or in the presence of 10 μM amastatin and to human thrombin. Each point represents mean ± S.E.M of 4 observations. The platelet rich plasma was incubated for 2 min with amastatin (10 μM) before challenge with 100 μM TRAP.

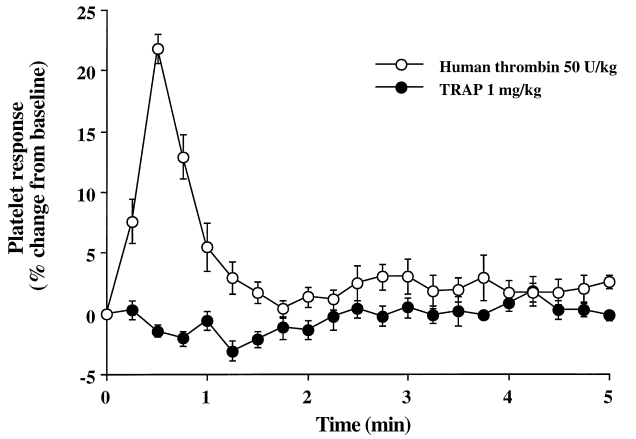

Fig. 2. The time-course of changes in intrapulmonary platelet accumulation in response to TRAP and human thrombin in rats. Each point represents the average of 6–8 observations.

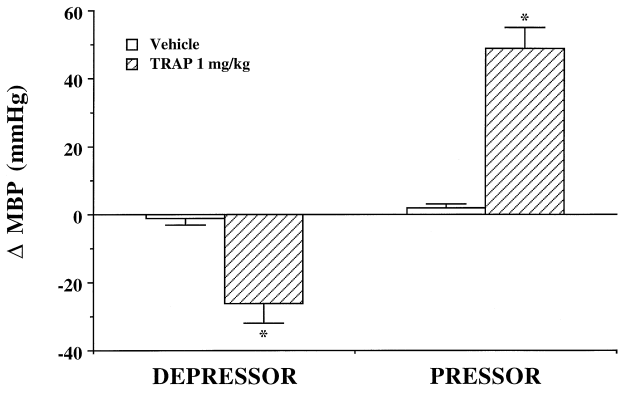

Fig. 4. Summary of the depressor and pressor responses to TRAP in rats (mean ± S.E.M, $n = 8$). $* p < 0.01$ vs. vehicle (ANOVA and Scheffe F-test). Baseline mean blood pressure in these rats were 100 ± 4 mmHg.

shown). In contrast TRAP failed to cause a shape change or aggregation in rat platelet rich plasma up to 300 μM (Fig. 1). Reports in the literature have suggested that aminopeptidase M in human platelet rich plasma can inactivate TRAPs by cleaving the N-terminal serine (Coller et al., 1992). Hence we pre-incubated rat platelet rich plasma with 10 μM amastatin, an inhibitor of aminopeptidase M. Under these conditions TRAP still failed to induce aggregation in rat platelet rich plasma. Human thrombin (0.1 U ml^{-1}) on the other hand caused pronounced platelet aggregation in rat platelet rich plasma (Fig. 1). In addition pretreatment of rat platelets with TRAP (100 μM) did not alter thrombin induced aggregation suggesting a lack of cross desensitization (85 ± 3% vs. 87 ± 6% aggregation in response to 0.1 U/ml thrombin in the absence and presence of 100 μM TRAP). The rat sequence peptide SFFLRN (10, 30 and 100 μM) failed to induce platelet aggregation in rat platelet rich plasma (3 ± 2, 2 ± 2 and 4 ± 2% aggregation respectively, $n = 3$ for each dose).

3.2. Platelet aggregation in vivo

TRAP (1 mg/kg, i.v., bolus) failed to cause intrapulmonary platelet aggregation in the rat in vivo (Fig. 2). TRAP at a higher dose (2 mg/kg, i.v, bolus) also failed to induce aggregation (data not shown) but was associated with very pronounced changes in blood pressure. Thus no higher doses were evaluated. In contrast human thrombin (50 U/kg, i.v.) induced a robust aggregatory response which was reversible (Fig. 2). Peak aggregation occurred 30 s after thrombin administration and the response returned to baseline within 2 min.

3.3. Blood pressure responses in rats

TRAP (1 mg/kg, i.v., bolus) in rats produced a biphasic response in blood pressure characterized by an initial depressor response (−25 ± 3 mmHg) lasting 15–30 s followed by a pronounced pressor response (50 ± 7 mmHg) that lasted for 2–3 min. A representative tracing of the blood pressure response elicited by TRAP is shown in Fig. 3 and data summarized in Fig. 4. In the pithed rats the depressor response to TRAP was abolished while the pressor response was significantly augmented (49 ± 1 vs. 71 ± 4 mmHg, before and after pithing respectively, Fig. 5). In the nephrectomized rats the the blood pressure responses were not altered (−24 ± 3 and 55 ± 5 vs. −21 ± 4 and 66 ± 8, depressor and pressor responses before and after bilateral nephrectomy respectively). In separate groups of rats, pretreatment with L-NAME (0.3 mg/kg per min × 30 min, i.v.) or indomethacin (3 mg kg^{-1}, i.v.) did

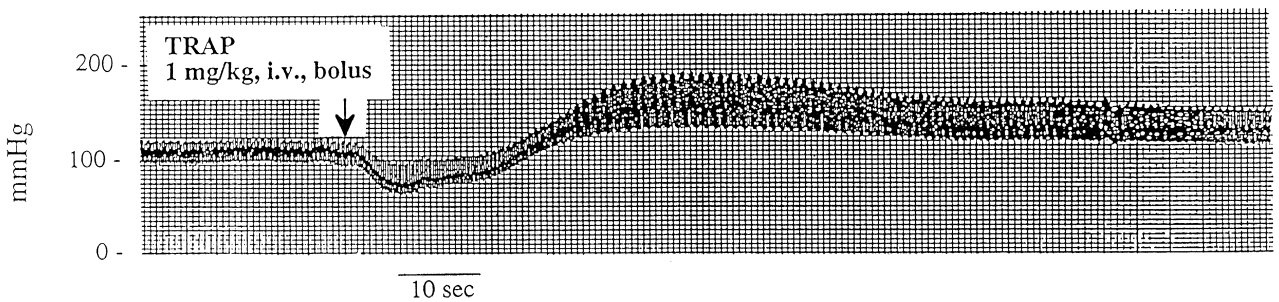

Fig. 3. Representative tracing of the blood pressure responses to TRAP in rats.

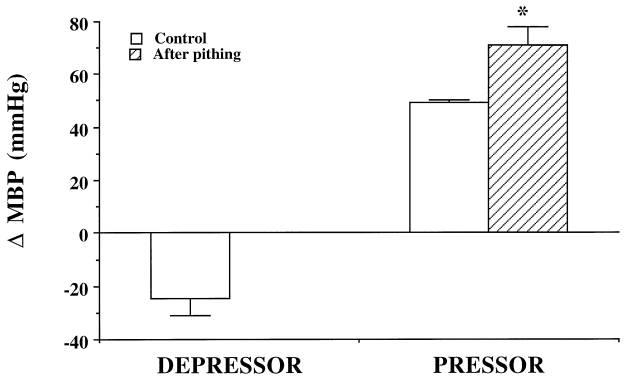

Fig. 5. Summary of the depressor and pressor responses to TRAP before (control) after pithing in the same group of rats (top panel, mean ± S.E.M, $n = 5$). * $p < 0.01$ vs. the changes in the control period (ANOVA and Scheffe *F*-test). Baseline mean blood pressure (mmHg) before and after pithing were 120 ± 7 and 48 ± 6 respectively.

not alter the blood pressure response to TRAP (−27 ± 3 and 49 ± 4 vs. −24 ± 3 and 53 ± 5 for the L-NAME group, and −24 ± 1 and 49 ± 3 vs. −26 ± 2 and 56 ± 4 for the indomethacin group; depressor and pressor responses before and after drug respectively). Pretreatment with thrombin (50 U/kg) failed to attenuate the blood pressure response to TRAP suggesting a lack of desensitization (−22 and 45 vs. −19 and 51 mmHg: depressor and pressor responses before and after thrombin respectively, $n = 2$). Higher doses of thrombin were not tested because of the profound platelet aggregation response in pulmonary microcirculation.

3.4. Hemodynamic effects of TRAP in rats

TRAP (1 mg/kg, i.v., bolus) caused significant increases in blood pressure and heart rate (Table 1). Cardiac output was unchanged, thus the calculated total peripheral resistence was significantly increased (Table 1). These hemodynamic changes were obtained 1–2 min after i.v. injections of TRAP to coincide with the peak pressor response. Since the depressor responses were very transient (15–30 s) we did not attempt to determine the hemodynamics during this stage of the blood pressure response.

4. Discussion

Rats respond to TRAP with marked changes in blood pressure, most likely a result of direct vascular action mediated by thrombin receptor activation. The vascular effects of TRAP can not be attributed to any secondary effects derived intrapulmonary from platelet aggregation, as the rat platelets are resistant to TRAP.

In the intrapulmonary model of platelet accumulation using ^{111}indium-labeled platelets, we and others have shown that rats, rabbits and guinea pigs are sensitive to platelet agonists (Chiu and Tetzloff, 1994; Chiu et al., 1997; Oyekan and Botting, 1986; Sandoli et al., 1994). In this rat model TRAP failed to induce platelet aggregation consistent with in vitro results. TRAP at this dose was associated with substantial changes in blood pressure and was therefore not tested at higher doses. In contrast, human thrombin produced a robust platelet aggregatory response which was accompanied by a fall in blood pressure (−42 ± 7 mmHg). This hypotensive response can be attributed to the hypoxia associated with platelet aggregates in the pulmonary microcirculation in response to agonists (Klee and Seiffge, 1991); a phenomenon we have observed with other platelet agonists such as collagen and ADP.

Intravenous injection of TRAP in rats produced a biphasic response in blood pressure characterized by an initial depressor response followed by a more pronounced pressor response. These changes in blood pressure suggest that the rat vasculature is responsive to TRAP. Several investigators using cell culture as well as in vitro systems have reported that TRAP can cause the release of EDRF and prostacyclin (Awbrey et al., 1979; Tesfamariam et al., 1993). TRAP-induced relaxation of rat aortic rings was abolished by pretreatment of the tissues with L-NAME (an inhibitor of EDRF synthesis) and indomethacin (an inhibitor of prostacyclin synthesis). We hypothesized that the depressor response to TRAP could be mediated by the release of EDRF or of prostacyclin. However, we were

Table 1
Hemodynamic effects of TRAP (1 mg/kg, i.v., bolus) in inactin-anesthetized normotensive rats

Measurements	Vehicle		TRAP (1 mg/kg)	
	Baseline	Maximum Δ	Baseline	Maximum Δ
Mean blood pressure (mmHg)	131 ± 4	−1 ± 1	120 ± 6	+59 ± 2[a]
Heart rate (beats/min)	404 ± 16	+6 ± 6	363 ± 15	+80 ± 18[a]
Cardiac output (ml/min)	144 ± 5	+14 ± 5	148 ± 15	0 ± 26
Stroke volume (μl/min)	358 ± 18	+27 ± 14	408 ± 40	−78 ± 66
Total peripheral resistance (PRU)	917 ± 56	−86 ± 23	838 ± 92	+444 ± 231[a]

All data represents mean ± S.E.M ($n = 5$ for each group). All measurements were made 1 min following injection of TRAP or vehicle.
[a] $P < 0.05$, ANOVA and Scheffe's *F*-test from the corresponding change in the vehicle-treated rats.

unable to attenuate the depressor response to TRAP by L-NAME or indomethacin. In pilot studies this dose of L-NAME abolished the depressor response to acetylcholine, an agent that induces the release of EDRF (data not shown). Furthermore in our studies L-NAME infusion caused a significant increase in basal mean blood pressure (115 ± 4 vs. 138 ± 3 mmHg before and after L-NAME infusion) suggesting that a substantial inhibition of endogenous EDRF synthesis was achieved. Indomethacin also did not inhibit the depressor response to TRAP at a dose that is sufficient to inhibit endogenous prostacyclin synthesis. These results are in marked contrast to the in vitro studies reported in the literature showing inhibition by L-NAME and indomethacin to the relaxation induced by TRAP (Antonaccio et al., 1993; Tesfamariam et al., 1993). The reason for this discrepancy is not clear. It is possible that TRAP may be causing the release of other vasodilators such as endothelial derived hyperpolarising factor. Alternatively, a transient decrease in cardiac output by TRAP could account for the depressor phase of the biphasic blood pressure response in vivo. In a recent report in dogs TRAP was shown to decrease cardiac output when infused into the circumflex coronary artery (Damiano et al., 1996).

In the hemodynamic studies, TRAP caused a substantial increase in total peripheral resistence which could account for the pressor response. Cardiac output was unchanged in these studies and heart rate was significantly elevated after TRAP. This substantial increase in heart rate observed in these studies may be a direct consequence of thrombin receptor activation or secondary to any coronary vasoconstriction. To determine if the pressor response to TRAP was due to a direct effect of TRAP in causing smooth muscle contraction we employed the pithed rat model since blood pressure in intact animals is controlled by both central and peripheral mechanisms. In the pithed rat the central control of blood pressure is essentially destroyed and the basal blood pressure is very low (48 ± 6 vs. 120 ± 7 mmHg respectively for mean blood pressure in pithed vs. intact animals) indicating the lack of sympathetic tone. This model is reported to be sensitive to vasoactive agents (Grant and McGrath, 1988) and under these conditions only the pressor but not the depressor response to TRAP was obtained. The pressor responses were significantly elevated after pithing (49 ± 1 vs. 71 ± 4 mmHg, before and after pithing respectively). This effect may be attributed to the absence of the depressor response or the compound may have elicited a more pronounced vasoconstriction in the presence of a low basal vascular tone. Nonetheless, these observations suggest that the effects of TRAP are due to a direct effect on the vascular smooth muscle. The lack of a depressor response to TRAP after pithing may be due to maximally expressed vasodilator mechanisms.

The thrombin receptor mRNA was shown to be highly expressed in the rat kidney (Zhong et al., 1992). Hence we used nephrectomized rats to determine the contributions of the kidneys to the pressor responses to TRAP. The pressor responses were not altered in the nephrectomized rats. These results indicate that the increase in TPR was not significantly derived from renal vasoconstriction.

The central role of thrombin in inducing platelet aggregation has been recognized. These platelet effects of thrombin have been mimicked by TRAPs suggesting that thrombin mainly exerts its effects via interactions between proteolytically generated-tethered ligand and its receptor. Blockade of the platelet thrombin receptor with a polyclonal antibody (IgG 9600) raised against a peptide derived from the thrombin-binding exosite region of the cloned human thrombin receptor, has been shown to effectively inhibit platelet-dependent cyclic flow reductions in the carotid artery of the African green monkey (Cook et al., 1995). However, a recent study demonstrated that adult mice with disruption of the thrombin receptor gene were not associated with bleeding diathesis (Connolly et al., 1996). These findings suggest that the cloned thrombin receptor is important for platelet function in man, monkeys and guinea pigs but not in lower species including rats and mice. In rats, the cloned thrombin receptor appears to mediate vascular contraction; a second thrombin receptor may mediate the platelet function. In this context it should be pointed out that platelets from thrombin receptor gene knock out mice have demonstrated a strong aggregatory response to thrombin presumably mediated by a second thrombin receptor not yet identified (Connolly et al., 1996).

The vascular and hemodynamic responses observed in the present studies following intravenous injections of TRAP may not be physiological since thrombin is not a circulating factor and the activity of thrombin under normal conditions is tightly controlled. However, it is conceivable that thrombin, generated at a site of vascular injury, could cause local vasoconstriction and promote thrombus growth via this thrombin receptor-mediated response. Blockade of the thrombin receptor with selective thrombin receptor antagonists under these conditions may have potential clinical utility. The lack of a platelet response makes the rat a good model to study the vascular effects of TRAPs and to evaluate the potential benefit of thrombin receptor antagonists in vascular disorders without activating the platelets.

References

Antonaccio, M.J., Normandin, D., Serafino, R., Moreland, S., 1993. Effects of thrombin and thrombin receptor activating peptides on rat aortic vascular smooth muscle. J. Pharmacol. Exp. Ther. 266, 125–133.

Awbrey, B., Hoak, J., Owen, W., 1979. Binding of human thrombin to cultured endothelial cells. J. Biol. Chem. 254, 4092–4095.

Bar-Shavit, R., Benezra, M., Eldor, A., Hy-Am, E., Fenton, J.W. II, Vlodavsky, I., 1990. Thrombin immobilized to extracellular matrix is a potent mitogen for vascular smooth muscle cells: nonenzymatic mode of action. Cell. Regul. 1, 453–463.

Baum, T., Sybertz, E.J., Watkins, R.W., Nelson, S., Coleman, W., Pula, K.K., Prioli, N., Rivelli, M., Gossman, A., 1986. Hemodynamic actions of a synthetic atrial natriuretic factor. J. Cardiovasc. Pharmacol. 8, 898–904.

Chen, L., Buchanan, J., 1975. Mitogenic activity of blood components I, Thrombin and prothrombin. Proc. Natl. Acad. Sci. USA 72, 131–135.

Chiu, P.J.S., Tetzloff, G., 1994. EDRF (NO)-mediated modulation of collagen-induced platelet accumulation in rat pulmonary microcirculation. J. Biomed. Sci. 1, 43–48.

Chiu, P.J.S., Tetzloff, G., Foster, C., Chintala, M.S., Sybertz, E.J., 1997. Characterization of in vitro and in vivo platelet responses to thrombin and thrombin receptor activating peptides in guinea pigs. Eur. J. Pharmacol. 321, 463–469.

Coller, B.S., Ward, P., Ceruso, M., Scudder, L.E., Springer, K., Kutok, J., Prestwich, G.D., 1992. Thrombin receptor activating peptides, Importance of the N-terminal serine and its ionization state as judged by pH dependence, nuclear magnetic resonance spectroscopy, and cleavage by aminopeptidase M. Biochemistry 31, 11713–11720.

Connolly, T.M., Condra, C., Feng, D.-M., Cook, J.J., Stranieri, M.T., Reilly, C.F., Nutt, R.F., Gould, R.J., 1994. Species variability in platelet and cellular responsiveness to thrombin receptor-derived peptides. Thromb. Haemost. 72, 627–633.

Connolly, A.J., Ishihara, H., Kahu, M.K., Farese, R.V., Coughlin, S.R., 1996. Role of the thrombin receptor in development and evidence for a second receptor. Nature 381, 516–519.

Cook, N.S., Zerwes, H.-G., Tapparelli, C., Powling, M., Singh, J., Metternich, R., Hagenbach, A., 1993. Platelet aggregation and fibrinogen binding in human, rhesus monkey, guinea-pig, hamster and rat blood: Activation by ADP and a thrombin receptor peptide and inhibition by glycoprotein IIb/IIIa antagonists. Thromb. Haemost. 70, 531–539.

Cook, J.J., Sitko, G.R., Bednar, B., Condra, C., Melott, M.J., Feng, D.-M., Nutt, R.F., Shafer, J.A., Gould, R.J., Collony, T.M., 1995. An antibody against the exosite of the cloned thrombin receptor inhibits experimental arterial thrombosis in the African green monkey. Circulation 91, 2961–2971.

Damiano, B.P., Cheung, W., Mitchell, J.A., Falotico, R., 1996. Cardiovascular actions of thrombin receptor activation. J. Pharmacol. Exp. Ther. 279, 1365–1378.

Dohlman, H.G., Thorner, J., Caron, M.G., Lefkowitz, R.J., 1991. Model systems for the study of seven-transmembrane segment receptors. Annu. Rev. Biochem. 60, 635–688.

Fenton, J.W. II, 1988. Regulation of thrombin generation and functions. Semin. Thromb. Hemost. 14, 234–240.

Gebremedhin, D., Ballagi-Pordany, G., Hadhazy, P., Magyar, K., Machovich, R., 1986. Species specificity of thrombin-induced changes in vascular tone. Eur. J. Pharmacol. 132, 71–74.

Gilman, A.C., 1987. G proteins, transducers of receptor-generated signals. Annu. Rev. Biochem. 56, 615–649.

Glusa, E., 1992. Vascular effects of thrombin. Semin. Thromb. Hemost. 18, 296–304.

Grant, T.L., McGrath, J.C., 1988. Interaction between angiotensin II and alpha adrenoceptor agonists mediating pressor responses in the pithed rat. Br. J. Pharmacol. 95, 1229–1240.

Huang, R., Sorisky, A., Church, W.A., Simons, E.R., Rittenhouse, S.E., 1991. 'Thrombin' receptor-directed ligand accounts for the activation by thrombin of platelet phospholipase C and accumulation of 3-phosphorylated phosphopeptides. J. Biol. Chem. 266, 18435–18438.

Klee, A., Seiffge, D., 1991. Evaluation of pulmonary accumulation of 51 chromium-labelled rat platelets following intravenous application of ADP and collagen. Thromb. Haemost. 65, 588–594.

Kinlough-Rathbone, R.L., Rand, M.L., Packham, M.A., 1993. Rabbit and rat platelets do not respond to thrombin receptor peptides that activate human platelets. Blood 82, 103–106.

Ku, D.D., Zaleski, J.K., 1993. Receptor mechanism of thrombin-induced endothelium-dependent and endothelium-independent coronary vascular effects in dogs. J. Cardiovasc. Pharmacol. 22, 609–616.

Lum, H., Anderson, T.T., Fenton II, J.W., Malik, A.B., 1994. Thrombin receptor activation peptide induces pulmonary vasoconstriction. Am. J. Physiol. 266, 448–454.

Oyekan, A.O., Botting, J.H., 1986. A minimally invasive technique for the study of intravascular platelet aggregation in anesthetized rats. J. Pharmacol. Methods 15, 271–277.

Rapoport, R.M., Draznin, M.B., Murad, F., 1984. Mechanisms of adenosine triphosphate, thrombin, and trypsin-induced relaxations of rat thoracic aorta. Circ. Res. 55, 468–479.

Sandoli, D., Chiu, P.J.S., Chintala, M., Dionisotti, S., Ongini, E., 1994. Effect of A_1 and A_2 adenosine receptor agonists on ex vivo and in vivo platelet aggregation in the rabbit. Eur. J. Pharmacol. 259, 43–50.

Tesfamariam, B., Allen, G.T., Normandin, D., Antonaccio, M.J., 1993. Involvement of the 'tethered ligand' receptor in the thrombin-induced endothelium-dependent relaxations. Am. J. Physiol. 265, 1744–1749.

Vassalo, R.R. Jr., Keiber-Emmons, T., Cichowski, K., Brass, L.F., 1991. Structure-function relationships in the activation of platelet thrombin receptors by receptor-derived peptides. J. Biol. Chem. 267, 6081–6085.

Vu, T.-K.H., Hung, D.T., Wheaton, V.I., Coughlin, S.R., 1991. Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. Cell 64, 1057–1068.

Zhong, C., Hayzer, D.J., Corson, M.A., Runge, M.S., 1992. Molecular cloning of the rat vascular smooth muscle thrombin receptor. Evidence for in vitro regulation by basic fibroblast growth factor. J. Biol. Chem. 267, 16975–16979.