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Evidence for the presence of a protease-activated receptor distinct from the thrombin receptor in human keratinocytes

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ABSTRACT **Thrombin receptor activation was explored in human epidermal keratinocytes and human dermal fibroblasts, cells that are actively involved in skin tissue repair. The effects of thrombin, trypsin, and the receptor agonist peptides SFLLRN and TFRIFD were assessed in inositolphospholipid hydrolysis and calcium mobilization studies. Thrombin and SFLLRN stimulated fibroblasts in both assays to a similar extent, whereas TFRIFD was less potent. Trypsin demonstrated weak efficacy in these assays in comparison with thrombin. Results in fibroblasts were consistent with human platelet thrombin receptor activation. Keratinocytes, however, exhibited a distinct profile, with trypsin being a far better activator of inositolphospholipid hydrolysis and calcium mobilization than thrombin. Furthermore, SFLLRN was more efficacious than thrombin, whereas no response was observed with TFRIFD. Since our data indicated that keratinocytes possess a trypsin-sensitive receptor, we addressed the possibility that these cells express the human homologue of the newly described murine protease-activated receptor, PAR-2 [Nystedt, S., Emilsson, K., Wahlestedt, C. & Sundelin, J. (1994) *Proc. Natl. Acad. Sci. USA* 91, 9208–9212]. PAR-2 is activated by nanomolar concentrations of trypsin and possesses the tethered ligand sequence SLIGRL. SLIGRL was found to be equipotent with SFLLRN in activating keratinocyte inositolphospholipid hydrolysis and calcium mobilization. Desensitization studies indicated that SFLLRN, SLIGRL, and trypsin activate a common receptor, PAR-2. Northern blot analyses detected a transcript of PAR-2 in total RNA from keratinocytes but not fibroblasts. Levels of thrombin receptor message were equivalent in the two cell types. Our results indicate that human keratinocytes possess PAR-2, suggesting a potential role for this receptor in tissue repair and/or skin-related disorders.**

Tissue repair involves a series of cellular responses including platelet-dependent primary hemostasis, formation of fibroblast-rich granulation tissue, and re-epithelialization due to migrating and proliferating basal keratinocytes (1). During cutaneous injury, activation of the coagulation cascade leads to the generation of the serine protease thrombin (1). The action of thrombin in response to injury may not be limited to its effects on hemostasis, inasmuch as thrombin can also stimulate fibroblasts to increase cell proliferation and collagen synthesis (2–4). However, the effect of thrombin on keratinocytes is not well characterized. Since thrombin stimulates inositolphospholipid (phosphoinositide) hydrolysis in cultured keratinocytes, a role in keratinocyte function is indicated (5). Also, the recent identification of thrombomodulin, a thrombin-binding surface glycoprotein that enhances thrombin's anticoagulant properties, on differentiated keratinocytes suggests a potential role for thrombin in the physiological regulation of keratinocyte maturation (6, 7).

Thrombin interacts with and activates a specific G-protein-coupled receptor (8). Activation of the thrombin receptor involves proteolytic cleavage of the N-terminal extracellular domain to unmask a new amino terminus, SFLLRN, which serves as a tethered peptide ligand. Amino acid sequences of functional thrombin receptors from human platelets and endothelial cells, Chinese hamster lung fibroblasts, rat vascular smooth muscle cells, and mouse osteoblastic cells have been derived from cDNA cloning and are highly homologous (9–12). In fact, synthetic peptides representing the tethered ligand peptide (e.g., SFLLRN) can initiate receptor activation independent of thrombin activity (13).

The thrombin receptor may be a prototype of a family of protease-activated receptors (14). A *Xenopus laevis* homologue of the human thrombin receptor with a strikingly different tethered ligand, TFRIFD (12), and a murine protease-activated receptor designated PAR-2 distinct from the murine thrombin receptor with specificity for trypsin over thrombin and a distinct tethered ligand, SLIGRL (15), have been reported.

In these studies, we explored the role of thrombin receptor activation in human keratinocytes and fibroblasts. After observing a significant enhancement of keratinocyte activation by trypsin compared with thrombin, we also evaluated the role of PAR-2 activation.

MATERIALS AND METHODS

Materials. Human α-thrombin was purchased from American Diagnostica (Greenwich, CT). SFLLRN-NH_2 and the scrambled control peptide FSLLRN-NH_2 were synthesized by The R. W. Johnson Pharmaceutical Research Institute (La Jolla, CA), and SLIGRL-NH_2 and TFRIFD-NH_2 were synthesized as described (13). These peptides are referred to as "SFLLRN," "FSLLRN," "SLIGRL," and "TFRIFD," respectively. All peptides possessed the amide carboxyl terminus. Bovine serum albumin (fraction V), bovine trypsin type 1 [9800 N^{α}-benzoyl-L-arginine ethyl ester (BAEE) units/mg of protein], Dulbecco's phosphate-buffered saline (PBS), and cell dissociation solution (CDS) were acquired from Sigma. Fura-2 acetoxymethyl ester (AM) was purchased from Molecular Probes. Dulbecco's modified Eagle's medium (DMEM), essential amino acids, and vitamins were obtained from Life Technologies (Grand Island, NY), and fetal bovine serum was from HyClone. [^{3}H]Inositol was purchased from American Radiolabeled Chemicals (St. Louis).

Cell Culture. Human neonatal keratinocytes were purchased from Cascade Biologics (Portland, OR) or Clonetics

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Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; $[Ca^{2+}]_i$, intracellular calcium concentration.

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(San Diego) as cryopreserved first-passage cells. Cells were grown in low calcium medium 154 with human keratinocyte growth supplement (bovine pituitary extract, bovine insulin, bovine transferrin, and human epidermal growth factor, KGM) from Cascade Biologics. Keratinocytes were grown to 60–80% confluence and subcultured by using trypsin/EDTA. Cells passaged 2–3 times were used in all experiments.

Human dermal fibroblasts (passage 8–11, AG1523B; Coriell Institute, Camden, NJ) were grown in DMEM supplemented with 20% fetal bovine serum, essential amino acids, and vitamins.

Measurement of [^{3}H]Inositol Phosphate Formation. Cells grown in 12-well dishes were labeled with 10 μCi of [^{3}H]inositol (20 Ci/mmol; 1 Ci = 37 GBq) for 48 hr. Cells were washed twice in PBS and then equilibrated in PBS containing 20 mM LiCl for 30 min at 37°C. Agonists were added and the cells were incubated for 15 min. Reactions were terminated by aspiration of the medium and addition of 750 μl of ice-cold 10 mM formic acid (16). Plates were chilled for 30 min and extracts were removed and diluted with 3 ml of 5 mM ammonium hydroxide.

Inositol phosphates were isolated by anion-exchange chromatography on columns containing 1 ml of Bio-Rad anion-exchange resin (AG1-X8, formate form, 200–400 mesh) as described (17). Data were expressed as net increase in dpm after subtraction of control values (dpm in the absence of agonist). The mean ± SEM for control values was 7906 ± 672 dpm and 17,300 ± 1697 dpm for fibroblasts and keratinocytes, respectively.

Intracellular Calcium. Human keratinocytes and human fibroblasts were removed from flasks by using a 15-min exposure to a nonenzymatic cell dissociation solution (CDS). Cells were washed twice in PBS with 1% bovine serum albumin (BSA) and cell number was adjusted to 1.5 to 2.0 × 10^6 viable cells per ml. Cells were labeled with 2 μM fura-2 acetoxymethyl ester at 37°C for 45 min in the dark. Labeled cells were washed twice in PBS with 0.2% BSA. Fluorescence measurements were recorded in a 2-ml aliquot for 5 min with stir setting on low by using a Perkin–Elmer LS-50B spectrofluorometer at 340 nm and 380 nm (excitation) and 510 nm (emission). Agonists were added at 30 sec from the start of recording. Data were collected as a ratio (R) of 340 nm/380 nm using the FL data manager software package (Perkin–Elmer). The maximal calcium response was determined by lysing the cells with 0.1% Triton X-100 (R_{max}), and the minimum calcium response (R_{min}) was determined by subsequent treatment of the cells with 20 mM Tris/10 mM EGTA, pH 7.2. Intracellular calcium concentrations were calculated for each sample over the 5-min recording period by using the formula $[Ca^{2+}]_i = K_d\ (R - R_{min})/R_{max} - R)$ with K_d = 224 nM (18). Desensitization studies were performed by activating cells with an agonist, followed 30 min later by addition of a second agonist. The peak calcium level (maximal calcium response minus baseline) was compared with that of the second agonist in the absence of an initial stimulus.

Analysis of PAR-2 and Thrombin Receptor mRNA on Keratinocytes and Fibroblasts. RNA was extracted from keratinocytes (60% confluent) or fibroblasts (90% confluent) by the Trizol method (Life Technologies). Poly(A) selection of mRNA by using oligo(dT)-cellulose (Collaborative Biomedical Products, Bedford, MA), Northern gel electrophoresis, transfer, and blot hybridizations were carried out according to the methods of Sambrook *et al.* (19). All probes used in this study were radiolabeled by random primer incorporation of [^{32}P]dCTP (GIBCO Life Technologies, Gaithersburg, MD). Sequences used as probes consisted of the human thrombin receptor, nucleotides 1–2123 (8) and murine PAR-2, nucleotides 373–805 (13), cDNAs. To obtain the 432-bp murine PAR-2 cDNA fragment, mouse embryonic total RNA was converted to cDNA with Superscript reverse transcriptase (GIBCO Life Technologies) by random priming. This cDNA (50 ng) was used as a template for the PCR. The murine PAR-2 primers used were 5′-GGGAAAGGGGTTCCGGTAGAACCAGGCTTTTCC-3′ and 5′-CGCCAACGGCGATGTTTGCCTTCTTCCTGGGG-3′ in 40 cycles of 30 sec at 94°C, 60 sec at 50°C, and 3 min at 72°C. The PCR product was subcloned by using the T/A cloning system (Invitrogen) and sequenced to confirm identity with murine PAR-2. Southern blotting experiments with the human thrombin receptor and murine PAR-2 cDNA fragments indicated that these two probes failed to cross-hybridize, indicating that hybridization signals were highly specific. Northern blots were normalized by probing with human glyceraldehyde-3-phosphate dehydrogenase

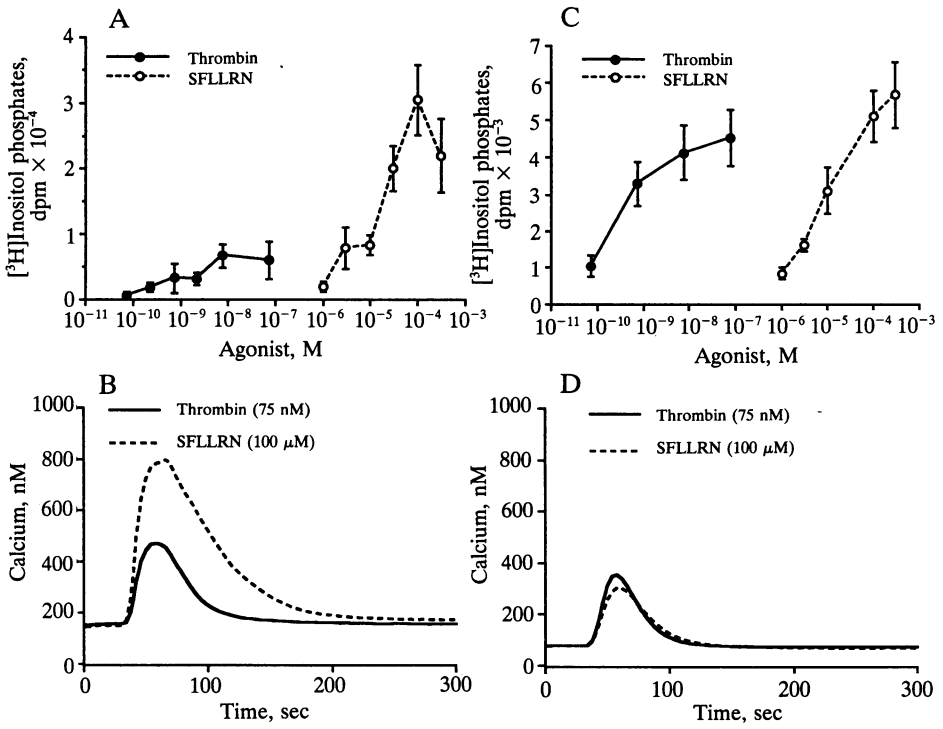

FIG. 1. Effects of thrombin and SFLLRN. The effects on inositol phosphate formation in keratinocytes and fibroblasts are shown in *A* and *C*, respectively. Data represent the mean ± SEM of a minimum of six independent experiments. Representative tracings of the $[Ca^{2+}]_i$ response to thrombin and SFLLRN in keratinocytes and fibroblasts over 5 min are shown in *B* and *D*, respectively. The agonists are administered as a single dose 30 sec after the onset of measurement. The tracings are typical of those observed in six to eight independent studies. Concentrations of thrombin and SFLLRN used in the calcium studies represent maximally effective doses based on experiments using 3- to 10-fold higher and lower concentrations of each agonist.

(GAPDH) (Clontech). Following hybridizations, blots were washed at 65°C twice in 2× SSC/0.1% SDS and twice in 0.2× SSC/0.1% SDS for 20 min per wash except for the GAPDH probe, which was washed for 1 hr. Films were exposed for 4 days at −70°C with intensifying screens.

RESULTS

Differential Effects of Thrombin and Thrombin Receptor Agonist Peptides in Keratinocytes and Fibroblasts. Thrombin receptor activation was evaluated in both keratinocytes and fibroblasts. Thrombin had little effect on inositol phosphate formation in keratinocytes at doses previously found to be maximally effective in inducing platelet aggregation (20). In contrast, SFLLRN induced inositol phosphate formation in keratinocytes dose dependently with an EC_{50} of 8.9 ± 1.9 μM (Fig. 1*A*). Thrombin (75 nM) induced an increase of $[Ca^{2+}]_i$ in keratinocytes; however, peak calcium concentration (192 ± 50 nM) was significantly less than that induced by 100 μM SFLLRN (517 ± 45 nM). A representative tracing of the calcium response is shown in Fig. 1*B*.

In contrast to keratinocytes, fibroblasts responded to thrombin and SFLLRN with EC_{50} values comparable to those reported for platelets (Fig. 1 *C* and *D*). Thrombin and SFLLRN induced inositol phosphate formation and calcium mobilization to similar extents, although the doses of SFLLRN required for receptor activation were greater than those for thrombin, as observed in other cell systems (4, 20, 21).

The effects of the *Xenopus* thrombin receptor tethered ligand peptide, TFRIFD, on inositol phosphate formation and $[Ca^{2+}]_i$ were also examined. TFRIFD has been reported to activate the human thrombin receptor (12). TFRIFD was minimally effective in inducing either inositol phosphate formation or a $[Ca^{2+}]_i$ increase in keratinocytes (Table 1 and Fig. 2). In fibroblasts, TFRIFD was capable of inducing inositol phosphate formation and $[Ca^{2+}]_i$ increase, albeit not as effectively as SFLLRN, consistent with previous reports (Table 1 and Fig. 2). In all assays and both cell types, the scrambled control peptide, FSLLRN, was evaluated at doses up to 300 μM a minimum of three times, and no response was observed.

Trypsin Activation in Keratinocytes. Calcium mobilization studies were carried out in which the keratinocytes were dissociated from plates by using trypsin instead of the nonenzymatic CDS. In these experiments, thrombin was unable to activate keratinocytes; however, SFLLRN maintained its effectiveness as an agonist (Fig. 3). Trypsin is a serine protease capable of cleaving the thrombin receptor (8). The effects of trypsin activation on inositol phosphate formation and $[Ca^{2+}]_i$ were further evaluated in keratinocytes. As stated earlier, thrombin was minimally effective in inducing inositol phosphate formation; in contrast, trypsin was extremely potent (Fig. 4). Furthermore, trypsin was found to be substantially more efficacious than thrombin in inducing calcium mobilization (Fig. 2).

Evidence for PAR-2 Activation in Keratinocytes. As these studies were being undertaken, evidence for the presence of a murine protease-activated receptor (PAR-2), which was shown to be highly responsive to trypsin (15) and is also apparently activated by a "tethered ligand" mechanism, was reported. The mouse PAR-2 tethered ligand sequence was identified as SLIGRL. Because our studies indicated that keratinocytes were more responsive to trypsin than to thrombin, we tested the effects of SLIGRL on inositol phosphate formation and $[Ca^{2+}]_i$ in keratinocytes and fibroblasts. SLIGRL was effective in inducing inositol phosphate formation in keratinocytes with an EC_{50} of 8.9 ± 1.9 μM, similar to that observed with SFLLRN. In contrast, SLIGRL was only minimally effective at doses up to 300 μM in fibroblasts (Fig. 5). Similarly, SLIGRL induced an increase in $[Ca^{2+}]_i$ in keratinocytes yet had no effect in fibroblasts (Fig. 2).

Table 1. Summary of EC_{50} values for inositol phosphate formation

Stimulus	Keratinocytes EC_{50}, nM	*n*	Fibroblasts EC_{50}, nM	*n*
Thrombin	ND	6	0.77 ± 0.36	7
Trypsin	4.3 ± 0.9	3	4.7 ± 1.1	3
SFLLRN	8300 ± 1100	14	7700 ± 1700	8
TFRIFD	ND	3	46,500 ± 12,100	4
SLIGRL	8900 ± 1900	3	ND	3

EC_{50} values were determined from concentration–response curves from *n* individual experiments. ND, unable to calculate an EC_{50} due to weak potency and efficacy.

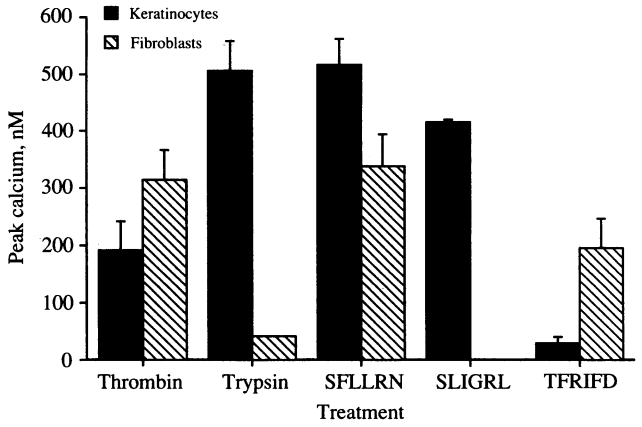

FIG. 2. Calcium mobilization (mean ± SEM peak calcium concentrations, nM) observed after stimulation of keratinocytes and fibroblasts with thrombin (75 nM), trypsin (27 nM), SFLLRN, SLIGRL, or TFRIFD (each at 100 μM). Each bar represents data from a minimum of three studies. Peak calcium levels were calculated by subtracting the baseline calcium levels from the maximal calcium responses to the agonists. Baseline calcium concentrations in all cell preparations were 75–120 nM.

Calcium Desensitization Studies in Keratinocytes. Activation of the thrombin receptor with either thrombin or SFLLRN results in a state of homologous or heterologous desensitization such that a second response to either thrombin or SFLLRN is diminished (22, 23). Desensitization experiments were carried out in the calcium mobilization assay to determine if the various agonists were activating PAR-2, thrombin receptor, or both receptors (Table 2). When keratinocytes were initially activated with trypsin, a secondary response to trypsin was virtually eliminated. Furthermore, the

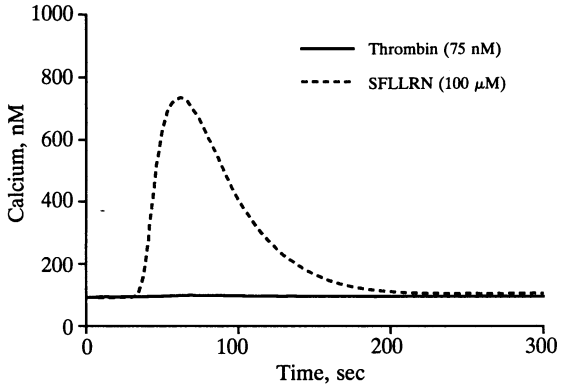

FIG. 3. Representative $[Ca^{2+}]_i$ tracing from keratinocytes exposed to a single dose of either thrombin or SFLLRN. The keratinocytes used in these studies were removed from culture plates by using trypsin/EDTA instead of CDS. The tracing indicates a complete loss of a thrombin response associated with trypsin/EDTA removal.

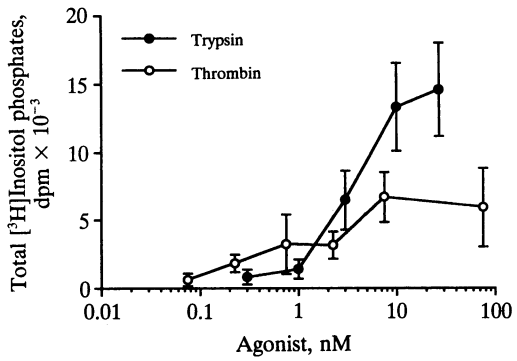

FIG. 4. Trypsin-induced inositol phosphate formation in keratinocytes. Data represent the mean ± SEM of three independent experiments. The thrombin data were taken from Fig. 1 and are shown here for comparative purposes.

calcium response to thrombin, SFLLRN, and SLIGRL was decreased by >50% after trypsin activation. Activation of keratinocytes with SLIGRL also resulted in a significant decrease in $[Ca^{2+}]_i$ subsequently induced by trypsin and SFLLRN. In addition, the keratinocyte response to SLIGRL, trypsin, and thrombin was significantly decreased after activation with SFLLRN. In contrast, pretreatment with thrombin was found to have no effect on secondary stimulation with either trypsin or SFLLRN. As expected, initial treatment with thrombin resulted in a complete lack of response to a secondary dose of thrombin. On the basis of these data, trypsin, SLIGRL, and SFLLRN appear to activate a common receptor, presumably PAR-2; trypsin and SFLLRN can activate both PAR-2 and thrombin receptor; and thrombin specifically activates its own receptor.

Detection of PAR-2 and Thrombin Receptor mRNA. RNA isolated from both fibroblasts and keratinocytes was subjected to Northern blot analysis to determine whether PAR-2 and/or thrombin receptor message is present in these cells. As shown in Fig. 6, fibroblasts express a 3.4-kb thrombin receptor transcript, in agreement with an earlier report (21). Our analysis indicated that keratinocytes contain comparable levels of the thrombin receptor transcript. When the murine PAR-2 probe was used, a 3.0-kb transcript that has the same relative mobility as the murine PAR-2 message (13) was detected in keratinocyte RNA. The murine PAR-2 transcript was not observed in fibroblast RNA, suggesting that PAR-2, if present, is expressed at a very low level in these cells. Even when poly(A)$^+$ RNA from fibroblasts was examined, PAR-2 transcript was not detected.

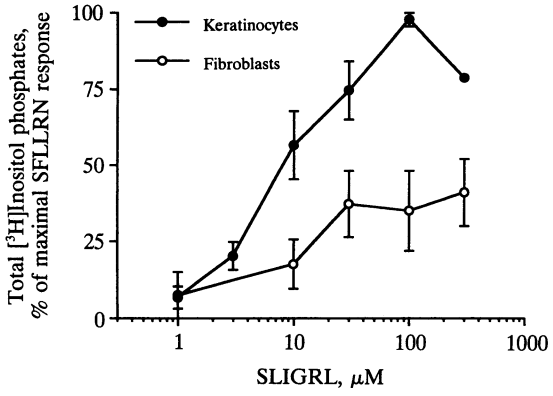

FIG. 5. Effect of SLIGRL on inositol phosphate formation. Data represent mean ± SEM from three individual experiments. The SLIGRL response was expressed relative to the maximal SFLLRN response to emphasize the cell specific responses to SLIGRL and SFLLRN.

Table 2. Desensitization of the calcium response in activated human keratinocytes

First agonist	Second agonist (at 30 min)	Desensitization, %
Trypsin	Trypsin	91.8 ± 4.8
Trypsin	Thrombin	74.1 ± 5.3
Trypsin	SFLLRN	52.8 ± 8.1
Trypsin	SLIGRL	56.5 ± 7.8
SLIGRL	Trypsin	78.1 ± 3.7
SLIGRL	SFLLRN	61.3 ± 18.0
SFLLRN	Thrombin	95.6 ± 1.9
SFLLRN	Trypsin	68.0 ± 6.9
SFLLRN	SLIGRL	64.3 ± 4.0
Thrombin	Thrombin	93.7 ± 0.4
Thrombin	Trypsin	<10
Thrombin	SFLLRN	<10

Doses of trypsin and thrombin used for desensitization studies were 27 and 75 nM, respectively. The dose of SFLLRN and SLIGRL was 100 μM. The percent desensitization was determined by measuring the peak calcium response (nM) of the second agonist expressed as the percentage change of the peak calcium response (nM) of that same agonist in keratinocytes not previously activated. Desensitization is expressed as mean ± SEM of a minimum of three studies carried out with different cell preparations.

DISCUSSION

The data presented demonstrate the presence of a protease-activated receptor on human keratinocytes. The receptor appears to be human PAR-2 on the basis of the following experimental evidence: (*i*) A peptide derived from the mouse PAR-2 tethered ligand sequence, SLIGRL, activates inositol phosphate formation and a $[Ca^{2+}]_i$ increase in keratinocytes. (*ii*) Trypsin, which has been reported to activate murine PAR-2, is extremely effective in activating inositol phosphate formation and $[Ca^{2+}]_i$ increase in keratinocytes, although the native protease for PAR-2 in keratinocytes is most likely not trypsin and has yet to be identified. (*iii*) A murine PAR-2 cDNA probe detects a transcript in RNA extracted from human keratinocytes. *Xenopus* oocytes expressing mouse or human PAR-2 respond to SFLLRN and SLIGRL as measured in calcium efflux experiments with nearly identical EC_{50}

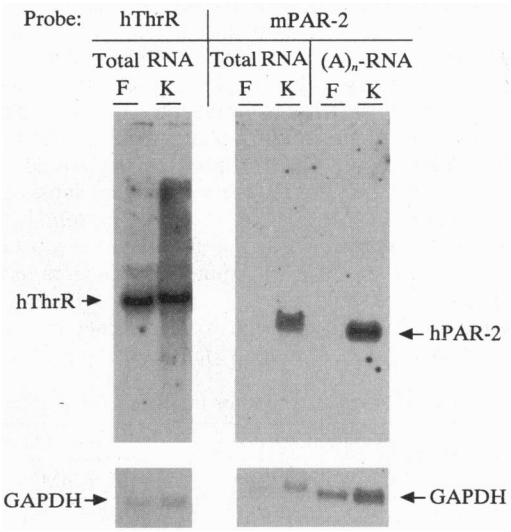

FIG. 6. Expression of PAR-2 and thrombin receptor mRNA in keratinocytes (K) and fibroblasts (F): Northern blot analysis of total RNA (20 μg) and poly(A)$^+$ RNA (4 μg) probed with human thrombin receptor (hThrR) or murine PAR-2 (mPAR-2) cDNA probes. After the exposure of the receptor cDNA probes, blots were rehybridized using a human GAPDH probe with a specific activity ≈25% of the receptor probes.

values. SLIGRL, however, is unable to activate human thrombin receptor expressed in oocytes (24). Our results in human keratinocytes are consistent with these observations in that SFLLRN is more efficacious than thrombin in inducing inositol phosphate formation and $[Ca^{2+}]_i$ increase. The fact that SFLLRN can activate PAR-2 is further supported by the desensitization studies in which trypsin and SLIGRL desensitize the SFLLRN $[Ca^{2+}]_i$ response and vice versa.

Despite the weak response of keratinocytes to thrombin, Northern blot analysis indicated the presence of thrombin receptor mRNA in keratinocytes at a level similar to that observed in fibroblasts. In addition, SFLLRN was found to be capable of desensitizing the keratinocyte $[Ca^{2+}]_i$ response to thrombin. The data presented here as well as results from *Xenopus* oocyte expression studies confirm that SFLLRN activates both PAR-2 and thrombin receptor on the same cell. Interestingly, the *Xenopus* tethered ligand sequence, TFRIFD, which has been shown to activate thrombin receptor in *Xenopus* oocyte expression experiments (12), was extremely weak in activating keratinocytes. In contrast, TFRIFD was a more effective agonist in fibroblasts. The weak activity of TFRIFD in keratinocytes is consistent with the relatively weak response to thrombin observed in keratinocytes and the inability of TFRIFD to activate PAR-2 expressed in *Xenopus* oocytes (24).

The function of the thrombin receptor in keratinocytes is unclear. Perhaps, while the amount of thrombin receptor RNA is similar to that of PAR-2 RNA in keratinocytes, the amount of protein may not be equivalent to PAR-2, as judged on the basis of functional aspects of receptor activation in our studies. We cannot rule out that thrombin receptors may exist in a cleaved state on the cell surface, be internalized, or be stored in intracellular organelles such as the Golgi complex (22, 23). Furthermore, the keratinocyte thrombin receptor may represent a closely related but distinct isoform.

Keratinocytes exist as mitotically active basal cells that eventually migrate outward and terminally differentiate to cornified cells that constitute the outermost layer of skin. Proper signaling to induce a timely process of proliferation, migration, differentiation, and apoptosis is crucial for proper skin formation and wound closure. The presence of both receptors in these cells may indicate involvement of protease-activated receptor-mediated signals that might be related to a particular cell function at a particular point in the differentiation pathway. In fact, previous reports indicate that protease inhibitors are capable of blocking apoptotic cell death (25). In addition, thrombomodulin has been localized in differentiating keratinocytes (6, 7). The lack of correlation between thrombin receptor mRNA levels and response to a thrombin receptor agonist could be explained by a temporal and regulated process of protein expression important for keratinocyte differentiation and/or function.

Our studies indicate that human fibroblasts express primarily the thrombin receptor. In fibroblasts, SLIGRL is not an effective agonist, but SFLLRN is as efficacious as thrombin. Furthermore, trypsin is only a weak agonist in human fibroblasts. Northern blot analysis failed to detect PAR-2 transcripts in either fibroblast total RNA or poly(A)$^+$ RNA. However, a low level of transcript was detected in reverse transcription/PCR using fibroblast total RNA and primers to murine PAR-2, indicative of trace amounts of PAR-2 mRNA in fibroblasts (data not shown).

The involvement of either or both of these receptors in wound repair or skin disorders needs clarification. Thrombin has been implicated in postclotting mechanisms that could lead to a role in regulating early and late stages of wound repair. Studies of the regulation and function of both thrombin receptor and PAR-2 in keratinocyte biology are required to better understand the involvement of these cells in various aspects of wound healing or skin pathology. Understanding how and if PAR-2 and thrombin receptor functionally interact within cells and tissues may provide important information in the general understanding of a whole class of proteolytically activated receptors (14).

M. F. Singer is a director of Johnson & Johnson.

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