Cardiovascular Responses Mediated by Protease-Activated Receptor-2 (PAR-2) and Thrombin Receptor (PAR-1) are Distinguished in Mice Deficient in PAR-2 or PAR-1

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ABSTRACT

We developed mice deficient in protease-activated receptor-2 (PAR-2) or PAR-1 to explore the pathophysiological functions of these receptors. In this report, we evaluated mean arterial pressure and heart rate (HR) changes in response to PAR-1 or PAR-2 activation in anesthetized wild-type (WT), PAR-1-deficient (PAR-1-/-), and PAR-2-deficient (PAR-2 -/-) mice. In WT mice, TFLLRNPNDK, a PAR-1 selective activating peptide, caused hypotension and HR decreases at 1 \( \mu \text{mol/kg} \). TFLLRN-PNDK also caused secondary hypertension following L-NAME pretreatment. These responses were absent in PAR-1 -/- mice.

In WT mice, SLIGRL, a PAR-2 selective activating peptide, caused hypotension without changing HR at 0.3 \( \mu \text{mol/kg} \). SLIGRL did not induce hypertension following \( \text{N}^\text{G} \)-nitro-L-arginine-methyl ester-HCl (L-NAME). The response to SLIGRL was absent in PAR-2 -/- mice. SFLLRN, a nonselective receptor activating peptide caused hypotension and HR decreases in WT mice at 0.3 \( \mu \text{mol/kg} \), as well as secondary hypertension following L-NAME. SFLLRN still induced hypotension in PAR-1 -/- mice, but HR decrease and secondary hypertension following L-NAME were absent. The hypotensive and bradycardic responses to SFLLRN and SFLLRN-PNDK in PAR-2 -/- mice were accentuated compared with WT mice. By using mouse strains deficient in either PAR-1 or PAR-2, we confirmed the in vivo specificity of TFLLRN and TFLLRN-PNDK as respective activating peptides for PAR-1 and PAR-2, and the distinct hemodynamic responses mediated by activation of PAR-1 or PAR-2. Moreover, the accentuated response to PAR-1 activation in PAR-2-deficient mice suggests a compensatory response and potential receptor cross-talk.

Protease-activated receptors belong to a novel emerging family of seven-transmembrane, G protein-coupled receptors that are activated by proteolysis. Because proteases are common enzymes in organisms and receptor activation is catalytic, such receptors represent an efficient means to modulate cellular responses. Thus far, only four receptors belonging to this family have been described. The thrombin receptor or protease-activated receptor-1 (PAR-1) is cleaved and activated by \( \alpha \)-thrombin (Vu et al., 1991) and mediates several cellular functions of thrombin such as induction of platelet aggregation (Vu et al., 1991), stimulation of cell proliferation (McNamara et al., 1993), and modulation of vascular tone (Muramatsu et al., 1992; Ku and Zaleski, 1993). Since the identification of PAR-1, three receptor homologs designated PAR-2, PAR-3, and PAR-4 have been identified. PAR-3 and PAR-4 are distinct proteolytically activated receptors for \( \alpha \)-thrombin (Ishihara et al., 1997; Kahn et al., 1998). PAR-2 was serendipitously identified by homology cloning of a mouse genomic library (Nystedt et al., 1994). Unlike PAR-1, PAR-3, and PAR-4, the physiologic activator of PAR-2 has not been identified. This has made elucidation of its potential physiologic or pathophysiologic roles more difficult. In vitro studies show that trypsin cleaves the extracellular amino terminus of PAR-2 to generate a tethered ligand and activate the receptor (Nystedt et al., 1994), analogous to PAR-1. Given the expression of PAR-2 in stomach and intestine (Nystedt et al., 1994, 1995), as well as the synthesis and secretion of trypsin in the gastrointestinal tract, trypsin activation of PAR-2 has been suggested to play a role in gastrointestinal pathophysiology (Kong et al., 1997). However, trypsin is unlikely to represent the physiologic agonist for PAR-2 in the vascular compartment and most extracellular spaces because of the absence of trypsin at these sites. Tryptase has also been reported to activate PAR-2 and may be a more likely

ABBREVIATIONS: PAR-1, protease-activated receptor-1; PAR-2 protease-activated receptor-2; MAP, mean arterial pressure; HR, heart rate; L-NAME, \( \text{N}^\text{G} \)-nitro-L-arginine-methyl ester-HCl.
proteolytic activator of PAR-2 (Corvera et al., 1997; Fox et al., 1997; Mirza et al., 1997; Molino et al., 1997a). Because mast cells release tryptase as part of an inflammatory response and PAR-2 is up-regulated by several inflammatory mediators such as tumor necrosis factor-α and interleukin-1, PAR-2 has been suggested to participate in inflammatory responses (Nystedt et al., 1996; Corvera et al., 1997). PAR-2 is expressed in vascular endothelial cells, where it mediates proliferation (Mirza et al., 1997) and nitric oxide release (Saieddine et al., 1996), and in vascular smooth muscle cells (Saieddine et al., 1996), where it appears to induce proliferation (Bono et al., 1997). Moreover, the accumulation of mast cells in human atherosclerotic lesions (Kaartinen et al., 1994; Jeziorska et al., 1997) may activate these vascular PAR-2 receptors, suggesting a potential role for this receptor in vascular injury and inflammatory responses.

An alternative approach to defining physiological functions of protease-activated receptors involves assessing the responses to receptor activation. Specific receptor activating peptides activate the receptor by mimicking the tethered peptide activating sequence contained within the receptor (Vu et al., 1991; Nystedt et al., 1994). This approach has revealed several functions of PAR-2 activation including vasodilatation of isolated vascular preparations (Saieddine et al., 1996), vasodilatation of coronary vasculature in isolated perfused hearts (Haertlein et al., 1996), and stimulation of contraction in isolated gastric tissue (Saieddine et al., 1996).

In addition, evidence in rats (Hwa et al., 1996; Emilsson et al., 1997) and mice (Cheung et al., 1998) have shown that activation of PAR-2 decreases arterial pressure, presumably as a result of arterial vasodilatation. This in vivo response is similar to that caused by PAR-1 activation in vivo. However, the PAR-1 response is more complex, consisting of an initial arterial pressure decrease followed by an arterial pressure increase (Cheung et al., 1998). This hemodynamic profile is consistent with isolated vascular tissue studies showing that α-thrombin and PAR-1 receptor activating peptides induce both vasodilatation and vasoconstriction (Muramatsu et al., 1992; Ku and Zaleski, 1993).

Differentiation of the specific PAR-1 and PAR-2 responses using receptor activating peptides is compromised because these peptides may not be selective (Blackhart et al., 1996). Moreover, these peptides may also activate as yet unidentified protease-activated receptors or elicit other responses not mediated by protease-activated receptors. Therefore, we developed and used mice deficient in PAR-2 or PAR-1 to define the specific cardiovascular responses mediated by PAR-2 and PAR-1 activation in vivo and the specificity of these receptor activating peptides. Arterial pressure and heart rate (HR) responses to i.v. infused SLIGRL, a PAR-2 selective peptide, TFLLRNPNDK, a PAR-1 selective activating peptide, and SFLLRN, a PAR-1 and PAR-2 receptor activating peptide, were assessed in wild type (WT) and PAR-1- and PAR-2-deficient mice.

Materials and Methods

Generation of PAR-1- and PAR-2-Deficient Mice

A 129/simian virus mouse genomic DNA fragment (≈14 kilobases (kb)) containing the PAR-2 gene was cloned and characterized by restriction mapping. A 1.3-kb EcoRI-HindIII fragment of the PAR-2 gene was prepared by polymerase chain reaction (PCR) using oligonucleotides 5′-GCATACACATCTGCAGACCG-3′ and 5′-GCTG-GTCTCAGAT TTCTTAAG-3′ and was placed 5′ of the neomycin-resistant gene cassette in the targeting construct. A 5.5-kb SauI-EcoRI DNA fragment covering part of exon 2 and the 3′ flanking region of the PAR-2 gene was placed 3′ of the neo cassette (Fig. 1A).

A herpes simplex virus thymidine kinase gene cassette was also placed at the 3′ end of the construct for negative selection (Fig. 1A). The construct DNA was introduced into E14 embryonic stem (ES) cells by electroporation. Transfected ES cells were subjected to drug selection using 400 μg/ml of G418 (Geneticin; Gibco/BRL, Gaithersburg, MD) and 2 μM gancyclovir (Cytosin; Syntex Corp, Palo Alto, CA). ES cells with the targeted gene were detected by PCR using the PAR-2 gene-specific oligonucleotide 5′-GTCTCCTTAA CGGAGGC CTTCTC-3′ and the neomycin-specific oligonucleotide 5′-TACCCGGTAGA ATGTCCTAGCAG-3′. Targeted ES cell clones were injected into C57BL/6J blastocysts. Chimeras were bred with C57BL/6J mice and germline transmission was obtained. PAR-2 heterozygous mice were bred and offspring were genotyped using PCR analyses of tail DNA (Fig. 1B) with the following 5′–3′ primers: mPAR-2 WT upper, CTGTGGCCTCTGCCTGTCG; mPAR-2 WT lower, GCCACTGTAGCCGTTA GGAAGC; and mPAR-2 neomycin-specific upper, GTGGGGGTGGGGTGGGATTAGA.

To confirm absence of PAR-2 RNA, RNA was isolated from kidney and small intestine of PAR-2+/−, PAR-2−/−, and PAR-2−/− mice, poly A (Gibco/BRL) and used for Northern blot analyses as previously described (Darrow et al., 1996). A 432-basepair PAR-2 probe probe
PAR-2 activating peptide, was administered as an i.v. bolus infusion by homologous recombination as described previously (Darrow et al., 1996).

Animal Preparation

All procedures involving the use of animals were performed in accordance with the Guide for the Care and Use of Laboratory Animals (1996) and the Animal Care and Use Committee, The R.W. Johnson Pharmaceutical Research Institute. A total of 32 male mice, 16 normal WT, 8 homozygous PAR-1-deficient mice (PAR-1\(^{-/-}\)), and 8 homozygous PAR-2-deficient mice (PAR-2\(^{-/-}\)) were used. Three mice heterozygous for each receptor (PAR-1\(^{+/-}\) and PAR-2\(^{+/-}\)) were also studied. However, the cardiovascular responses of these heterozygotes was indistinguishable from WT and therefore are not discussed in detail in this report.

Mice with different genotypes were evaluated randomly, with the investigator unaware of genotype. Mice, at least 4 months old and weighing 30 to 35 g, were initially anesthetized with isoflurane (1.25%). A tracheal cannula (PE-90) was implanted via a midline cervical incision. Using a rodent respirator (Harvard Apparatus, South Natick, MA), mice were ventilated with 95% O\(_2\)/5% CO\(_2\) containing 0.75% isoflurane at a tidal volume of 0.2 ml and 140 breaths/min. Body temperature was maintained at 38°C with a heating lamp and a proportional temperature controller (Yellow Springs Instrument, Yellow Springs, OH). Subdermal needle electrodes were inserted for recording lead II electrocardiogram. A Teflon catheter (AWG30 tubing) tapered at one end and filled with saline containing heparin (10 U/ml) was inserted into the right carotid artery and advanced to the thoracic aorta. The catheter was attached to a Statham P50 pressure transducer (Spectramed, Oxnard, CA) for recording arterial blood pressure. Another catheter, constructed from Micro-Renathane MRE-033 tubing (Braintree Scientific, Inc., Braintree, MA), was inserted into the right jugular vein for administration of drugs and peptides. Arterial pressure and ECG signals were displayed on a Gould chart recorder and digitized and analyzed with a Po-Ne-Mah HD5/16/SW Cardiovascular Analysis System (Gould Instruments, Valley View, OH). Systolic, diastolic and mean arterial pressures (MAP), as well as HR, were measured continuously throughout the study. The preparation was allowed to stabilize for at least 30 to 60 min before the start of the experiment.

Chemicals and Peptides

Receptor activating peptides, SLIGRL-NH\(_2\), SFLRRN-NH\(_2\), and TFLLRNPNDK-NH\(_2\), were synthesized at the R.W. Johnson Pharmaceutical Research Institute, San Diego, CA. N\(^\text{ω}\)-Nitro-L-arginine-methyl ester-HCl (L-NAME; Alexis Corporation, San Diego, CA) and receptor activating peptides were dissolved in saline and infused into the jugular vein. To deliver doses of peptides and compounds accurately and in a small volume, each dose was prepared in a 4-μl volume and then loaded into the venous catheter containing 15 μl of saline. Fifty microliters of saline was then infused over 1 min using an infusion pump (model 55–111, Harvard Apparatus).

Experimental Protocol

Following control measurements, SFLRRN-NH\(_2\), a PAR-1 and PAR-2 activating peptide, was administered as an i.v. bolus infusion at 0.3 μmol/kg. Arterial pressure and HR were monitored for 15 min. SLIGRL-NH\(_2\), a selective PAR-2 activating peptide, was then infused i.v. at 0.3 μmol/kg and the response monitored for 15 min. Then, TFLLRNPNDK-NH\(_2\), a receptor activating peptide that, unlike SFLRRN, is selective for PAR-1, was administered in a similar fashion at 1 μmol/kg in the same mouse. Fifteen minutes following the final peptide infusion, L-NAME, an inhibitor of nitric oxide synthesis, was infused i.v. at 30 mg/kg as a bolus. Five minutes after L-NAME administration, SFLRRN-NH\(_2\), SLIGRL-NH\(_2\), and TFLLRNPNDK-NH\(_2\) were administered at the same doses as described above. In three PAR-1\(^{-/-}\) and three PAR-2\(^{-/-}\) mice, higher doses of the respective receptor activating peptides, TFLLRNPNDK (10 μmol/kg) or SLIGRL (3 μmol/kg), were tested. In addition, in three WT and three PAR-2-deficient mice, the response to acetylcholine (1 μg/kg) and angiotensin (0.1 μg/kg) was evaluated before the start of the receptor activating peptide studies.

Data Analysis

Arterial pressure and ECG signals were acquired at an analog-to-digital sampling rate of 250 Hz. MAP and HR were determined over a 5-s sampling interval every 5 s. Baseline values were determined by averaging the measurements for 20 s before each treatment. All data are expressed as mean ± S.E. Statistical significance of the differences in the maximum responses in WT and PAR-2 and PAR-1 mice was determined using one-way ANOVA and Dunnet’s test. P values less than 0.05 were considered statistically significant.

Results

Characterization of PAR-2-Deficient Mice

Matings of heterozygous pairs of mice were carried out through four cycles. Litter size ranged from 6 to 12 offspring. Of 419 mice analyzed, 28.4% were WT (+/+), 54.9% were heterozygous (+/-), and 16.7% were homozygous (-/-), which represents a significant variation (p < .005) from the expected Mendelian ratio (25:50:25). Furthermore, 20.0% of all PAR-2\(^{-/-}\) mice were either stillborn or found dead within 48 h of birth in comparison to 8.4% and 6.4% of PAR-2\(^{+/-}\) and PAR-2\(^{-/-}\) mice, respectively (p < .005). Five pups from each genotype, which died within 48 h of birth, were analyzed for anatomical and histological defects. No abnormalities were observed in any of these mice. The cause of this mortality is currently unknown. The remaining animals proceeded to maturity apparently without incident. Surviving PAR-2\(^{-/-}\) mice appeared normal upon gross anatomical and histological analysis. In addition, mating of PAR-2\(^{-/-}\) males with PAR-2\(^{-/-}\) females resulted in normal litter size and offspring.

In contrast, matings between PAR-1\(^{-/-}\) mice occurred less frequently and generally produced much smaller litter sizes, as has been previously reported (Connolly et al., 1996; Darrow et al., 1996).

Baseline Hemodynamic Parameters in PAR-2-Deficient Mice

Baseline arterial pressure and HR were not significantly different in PAR-1- and PAR-2-deficient mice compared with WT mice (Table 1). Baseline arterial pressure and HR following L-NAME were also not significantly different in PAR-1- and PAR-2-deficient mice compared with WT mice (Table 1).

In addition, the hypotensive response to acetylcholine (1 μg/kg) and the hypertensive response to angiotensin (0.1 μg/kg) was not different in three WT mice (−17 ± 2 and +23 ± 10 mm Hg, respectively) and three PAR-2-deficient mice (−23 ± 5 and +30 ± 8 mm Hg, respectively). The lack of effect of PAR-1 deficiency on responses to angiotensin and acetylcholine have been reported previously using a slightly different protocol (Darrow et al., 1996).
Hemodynamic Responses to Receptor Activating Peptides in PAR-2- and PAR-1-Deficient Mice

Effects of SLIGRL. SLIGRL (0.3 μmol/kg), a selective PAR-2 receptor activating peptide, was used to assess the loss of PAR-2 receptor function. Recent studies have shown that i.v. SLIGRL causes a marked, dose-dependent hypotension in normal anesthetized mice without affecting HR (Cheung et al., 1998). The duration of the hypotensive response to SLIGRL tended to be somewhat greater than that caused by PAR-1 activation. The present results confirm this profile in WT mice (Fig. 2, left and Fig. 5). However, three of the eight WT mice had a brief period of increased HR. Pretreatment with L-NAME, an inhibitor of nitric oxide synthesis, reduced the duration of the hypotensive response without affecting the maximum decrease in arterial pressure. SLIGRL did not affect HR following L-NAME treatment (data not shown).

In contrast to the response in WT mice, SLIGRL had no effect on arterial pressure or HR in PAR-2−/− mice before or after L-NAME (Fig. 2, right and Fig. 5). In three PAR-2−/− mice, a 10-fold higher dose of SLIGRL (3 μmol/kg) had no effect on arterial pressure (data not shown). These results confirm the lack of expression of PAR-2 in the receptor-deficient mice and demonstrate that the hypotensive response to SLIGRL is due exclusively to activation of PAR-2. The response to SLIGRL in PAR-1−/− mice was similar to that in WT mice, including a slight increase in HR (Fig. 2, middle).

Effects of TFLLRNPNDK. TFLLRNPNDK is a selective PAR-1 receptor activating peptide (Blackhart et al., 1996). As was shown previously (Cheung et al., 1998) and confirmed in the present studies, PAR-1 activation with TFLLRNPNDK in anesthetized WT mice causes a biphasic response consisting of transient hypotension followed by a rapid return to baseline (Fig. 3, left and Fig. 5). After L-NAME treatment, the transient hypotension is followed by a small but consistent hypertensive response. Also, as was documented previously (Cheung et al., 1998), the arterial pressure response is accompanied by an immediate but transient decrease in HR (Figs. 3 and 5). In the present studies this bradycardia was relatively small and followed by a brief period of increased HR. Although the HR decrease was not significantly different from that in PAR-1−/− mice, in which the response was absent, previous studies have shown that higher doses of TFLLRNPNDK result in consistent and marked HR decreases (Cheung et al., 1998).

TFLLRNPNDK had no effect on arterial pressure or HR in PAR-1−/− mice (Fig. 3, middle and Fig. 5). In three PAR-1−/− mice, a 10-fold higher dose of TFLLRNPNDK (3 μmol/kg) had no effect on arterial pressure (data not shown).

### TABLE 1
Baseline hemodynamics in WT and PAR-1- and PAR-2-deficient mice before and after L-NAME

Baseline hemodynamic values are mean ± S.E. for n = 8 PAR-1-deficient mice (PAR-1−/−), n = 8 PAR-2-deficient mice (PAR-2−/−), and n = 16 WT mice. Parameters were measured immediately before infusion of first peptide before and 5 min after L-NAME (30 mg/kg). SAP, systolic arterial pressure; DAP, diastolic arterial pressure.

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<td>MAP (mm Hg)</td>
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<td>HR (bpm)</td>
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![Figure 2](https://example.com/figure2.png)

**Fig. 2.** Arterial pressure and HR changes in response to SLIGRL, a PAR-2 selective receptor activating peptide, in WT and PAR-1- and PAR-2-deficient mice. Values are mean changes in MAP (upper panels) or HR (lower panels) for 16 WT, 8 PAR-1-deficient mice (PAR-1−/−), and 8 PAR-2-deficient mice (PAR-2−/−). SLIGRL (0.3 μmol/kg) was infused i.v. over 60 s and the response monitored for 5 min. The response to SLIGRL was retested after L-NAME (30 mg/kg i.v.). Note absence of response to SLIGRL in PAR-2-deficient mice.
mice, a 10-fold higher dose of TFLLRNPNDK (10 μmol/kg) had no effect on arterial pressure or HR (data not shown). In PAR-2−/− mice, the arterial pressure response to TFLLRNPNDK, as well as the transient HR decrease, was qualitatively similar to that in WT mice except that the magnitude of the hypotensive response and transient HR decrease was significantly greater (Fig. 3, right and Fig. 5). The hypertensive response to TFLLRNPNDK following L-NAME in PAR-2−/− mice was not different from WT.

Effects of SFLLRN. SFLLRN is a PAR-1 receptor activating peptide derived from the six amino-terminal amino acids of the human PAR-1 tethered ligand. This peptide has been shown to cause a biphasic arterial pressure response and transient HR decrease in anesthetized mice similar to that induced by TFLLRNPNDK (Cheung et al., 1998). This profile is confirmed in the present studies (Fig. 4, left and Fig. 5). The HR response is relatively small, but our previous studies showed that higher SFLLRN doses produce significantly greater reductions in HR (Cheung et al., 1998). Although SFLLRN activates PAR-1, it is now known to also activate PAR-2 (Blackhart et al., 1996). Thus, the hypertensive response to SFLLRN in PAR-1−/− mice is not completely inhibited compared with the response in WT mice (Fig. 4, middle and Fig. 5). As expected, the SFLLRN-induced hypertensive response following L-NAME as well as transient HR decrease, which are mediated exclusively by PAR-1 activation, were completely absent in PAR-1−/− mice. In PAR-2−/− mice, SFLLRN caused hypotension, transient HR decrease, and delayed hypertension following L-NAME similar to that induced in WT mice (Fig. 4, right and Fig. 5). However, like TFLLRNPNDK, SFLLRN-induced hypotension and transient HR reduction were significantly greater in PAR-2−/− mice. The hypertensive response to SFLLRN in PAR-2−/− mice was not affected.

Discussion

Using mouse strains deficient in PAR-1 or PAR-2, we confirmed the specific vascular responses to PAR-1 or PAR-2 receptor activation in vivo: PAR-2 activation results exclusively in hypotension without consistent changes in HR. The hypotension is presumably mediated by vasodilatation. In contrast, PAR-1 activation induces both an initial hypotensive response and HR decrease followed by hypertension. These changes in arterial pressure reflect both a vasodilatation and vasoconstrictor response. Furthermore, the accentuated PAR-1 response in PAR-2−/− mice suggests possible compensatory changes in PAR-1 expression or intracellular signaling, or other changes downstream from PAR-1 receptor activation.

Our previous studies in WT anesthetized mice (Cheung et al., 1998) documented the nature of these distinct receptor-specific responses by evaluating the hemodynamic dose responses to i.v. TFLLRNPNDK and SLIGRL, specific receptor activating peptides for PAR-1 and PAR-2, respectively. However, these studies could not exclude the possibility that TFLLRNPNDK or SLIGRL might have other actions not involving PAR-1 or PAR-2. PAR-3 and PAR-4, two recently identified protease-activated thrombin receptors (Ishihara et al., 1997; Kahn et al., 1998), do not appear to be activated in vitro by SFLLRN or SLIGRL. However, their identification presents the possibility that activation of an as yet unidentified protease-activated receptor may contribute to in vivo responses to TFLLRNPNDK and SLIGRL. Moreover, the receptor activating peptides may have other actions not involving protease-activated receptors. The present studies, by revealing the complete absence of hemodynamic actions of SLIGRL in PAR-2-deficient mice and the complete absence of hemodynamic actions of TFLLRNPNDK in PAR-1-deficient mice, confirmed that these receptors mediate the specific vascular effects observed in these mice.
mice, provide direct evidence for the specificity of these receptor activating peptides in vivo and confirm the nature of the hemodynamic responses to PAR-1 and PAR-2 activation.

Our studies also report, for the first time, the successful disruption of the PAR-2 gene in mice. Normal litter sizes of heterozygous and homozygous matings, normal progression to maturity, and normal gross anatomical and histological appearances of PAR-2$^{−/−}$ mice suggests a lack of obvious impact of PAR-2 deficiency on normal development and physiology. This result is in contrast to previous studies of PAR-1-deficient mice in which partial embryonic lethality was noted (Connolly et al., 1996; Darrow et al., 1996). However, surviving PAR-1-deficient mice proceeded to maturity and showed no gross anatomical or histological changes (Connolly et al., 1996; Darrow et al., 1996). The lack of obvious phenotype in PAR-1- or PAR-2-deficient mice may result from compensatory responses that minimize the impact of PAR-2 or PAR-1 deficiency in development and normal physiology. The consequences of PAR-2 deficiency may only be revealed in various pathological states and in response to pathological stimuli. These possibilities will require further testing of PAR-2$^{−/−}$ and PAR-1$^{−/−}$ mice using various models of pathology.

The accentuated response to PAR-1 activation in PAR-2$^{−/−}$ mice may be a manifestation of a compensatory response to the PAR-2 receptor deficiency. For example, PAR-1 expression may be increased in PAR-2-deficient mice. However, we have no evidence for this at this time. Studies in human endothelial cells have shown that PAR-1-specific antisense oligonucleotides reduce the thrombin response but not the PAR-2 response (Mirza et al., 1996). Although the converse experiment was not done, this finding suggests that the receptors are not linked in some way and do not require coexpression for their activity. However, activation of PAR-2

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**Fig. 4.** Arterial pressure and HR changes in response to SFLRN, a nonselective receptor activating peptide, in WT and PAR-1- and PAR-2-deficient mice. Values are mean changes in MAP for 16 WT, 8 PAR-1-deficient mice (PAR-1$^{−/−}$), and 8 PAR-2-deficient mice (PAR-2$^{−/−}$). SFLRN (0.3 μmol/kg) was infused i.v. over 60 s and the response monitored for 5 min. The response to SFLRN was retested after L-NAME (30 mg/kg i.v.). Note inhibition of delayed hypertensive response following L-NAME and transient HR decrease in PAR-1$^{−/−}$ mice.

**Fig. 5.** Summary of peak arterial pressure and HR responses to receptor activating peptides in WT and PAR-1- and PAR-2-deficient mice. Values are mean ± S.E. of the maximum changes in MAP or HR from experiments presented in Figs. 2–4 (16 WT, 8 PAR-1$^{−/−}$, and 8 PAR-2$^{−/−}$ mice). SFLRN or SLIGRL (0.3 μmol/kg) or TFLRN (1 μmol/kg) were infused i.v. over 60 s. Peak MAP or HR decrease was determined during the first 120 s or 60 s, respectively, following peptide dosing; peak MAP increase was determined following L-NAME pretreatment (30 mg/kg) during the first 120 s following peptide dosing. $p < .05$, significantly different from WT response by ANOVA.
resulted in desensitization of both PAR-1 and PAR-2 (Mirza et al., 1996). Moreover, PAR-1 desensitization was associated with receptor internalization (Mirza et al., 1996). These results suggest receptor “cross-talk” between PAR-1 and PAR-2. With respect to the present findings, it is possible that a continuous low level of PAR-2 activation in vivo results in a certain rate of PAR-1 internalization. When PAR-2 is not expressed, tonic internalization rate is reduced, resulting in a greater expression of PAR-1. However, this hypothesis is highly speculative. The exaggerated response is not likely to be a result of overall alteration in vascular responsiveness because baseline arterial pressure and HR were not different in PAR-2−/− mice, and the arterial pressure responses to angiotensin and acetylcholine were not altered in PAR-2−/− mice compared with WT mice. It is interesting that the hypertensive component of the PAR-1 response is not increased in PAR-2 deficient mice. This may appear inconsistent with the suggestion of up-regulation of PAR-1 in PAR-2-deficient mice. However, the hypertensive component is relatively small, such that small changes in this response may be difficult to detect or may be masked by the opposing, more marked hypotensive actions of PAR-1 activation. It is also possible that the altered PAR-1 response does not involve changes at the receptor level but rather, may result from changes downstream from receptor activation such as intracellular signaling or alteration in the expression or activity of other mediators or receptors. Thus, alteration of autonomic regulation may also play a role, because the response to PAR-1 activation in vivo appears to have a significant vagal component (Damiano et al., 1996; Cheung et al., 1998).


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