Inhibition of platelet thromboxane receptor function by a thrombin receptor–targeted pepducin

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To the editor:

Pepducins are novel cell-permeable peptides that modulate G-protein signaling by targeting the third intracellular loop of G-protein-coupled receptors (GPCRs)[1]. Some of these pepducins inhibit thrombin-induced platelet activation by impairing the function of the protease-activated receptor-1 (PAR-1) and PAR-4. In a recent study, Covic *et al.*[2] reported that the infusion of pepducin P4pal-10, which is based on the sequence of PAR-4, prolonged bleeding time and inhibited platelet activation in mice. The authors also tested the effects of P4pal-10 on human platelets. They concluded that this pepducin selectively inhibits PAR-1- and PAR-4-dependent platelet activation, but does not affect non-GPCRs such as collagen receptors (GPVI and $\alpha_2\beta_1$), or other GPCRs such as thromboxane A_2 (TXA_2) receptors or ADP receptors ($P2Y_1$ and $P2Y_{12}$).

We were able to reproduce the inhibitory effects of P4pal-10 on aggregation of washed human platelets stimulated with the soluble PAR-4 agonist AYPGKF, the soluble PAR-1 agonist SFLLRN and thrombin. However, we also observed a significant ($P < 0.05$) inhibition of collagen-induced platelet aggregation by P4pal-10 (**Fig. 1a**). For specificity testing, Covic *et al.*[2] used ADP (5 M), the TXA_2 mimetic U46619 (20 M), collagen (20 mg/ml) and ristocetin (1 mg/ml). Although we suppose that 20 mg/ml collagen was a typographic error, even 20 g/ml collagen would be a high concentration for aggregation studies[3]. In our studies, P4pal-10 did not inhibit platelet aggregation induced by high concentrations of collagen (≥10 g/ml), but almost completely blocked platelet aggregation at low collagen concentrations (≤3 g/ml; **Fig. 1b**), when secondary feedback mechanisms including ADP and TXA_2 (both acting on GPCRs) are essential for platelet aggregation[3]. Thus, we hypothesized that P4pal-10 may also interfere with thromboxane receptors. The concentrations of U46619 used by Covic *et al.*[2] exceed the maximal effective concentration of that compound by about two log orders of potency[4]. In our studies, platelet aggregation, which was induced by lower concentrations of U46619 (≤1 M), was almost completely inhibited by P4pal-10 (**Fig. 1c**).

Although pepducins are valuable tools for the study of GPCR function, we conclude that P4pal-10 is not a specific inhibitor of PAR-1 and PAR-4, as it also interferes with other GPCRs such as the thromboxane receptors. Thus, careful specificity studies are needed before pepducins can be generally recommended for the study of specific receptors in the field of drug discovery[5].

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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Covic *et al.* reply:

We previously showed that the PAR-4-based pepducin P4pal-10 blocks PAR-4- and PAR-1-dependent signaling and aggregation in human and mouse platelets[2]. Stampfuss *et al.* now raise the possibility that P4pal-10 may also inhibit the response of thromboxane receptors[8] to low concentrations of a thromboxane receptor agonist. Thromboxane receptors activate $G_{12/13}$ and G_q signaling pathways in platelets[9]. Activation of $G_{12/13}$

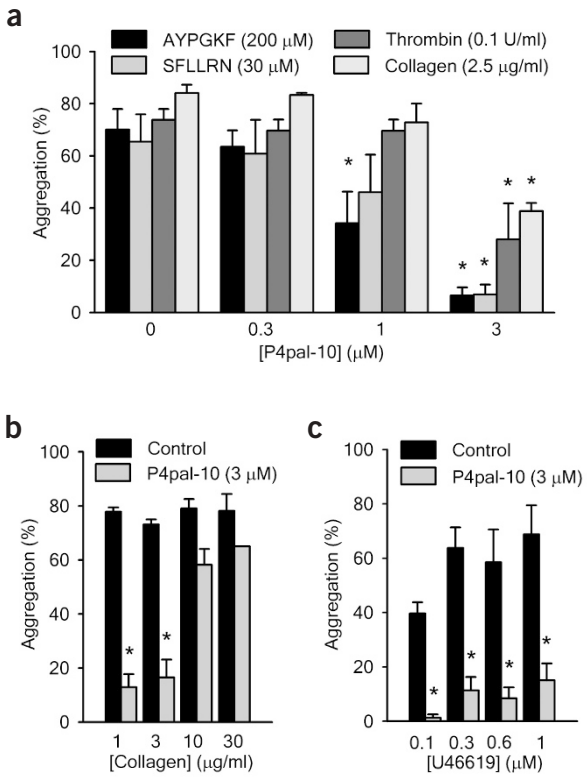

Figure 1 Effects of P4pal-10 on aggregation of washed human platelets. (**a**) P4pal-10 inhibits platelet aggregation induced by the soluble PAR-4 agonist AYPGKF, the soluble PAR-1 agonist SFLLRN, thrombin and collagen. (**b**) P4pal-10 inhibits platelet aggregation induced by low, but not high, concentrations of collagen. (**c**) P4pal-10 inhibits platelet aggregation induced by the TXA_2 mimetic U46619. Platelets were isolated and aggregation was measured turbidimetrically as previously described[6,7]. Data are shown as mean ± s.e.m. ($n = 4$). *, $P < 0.05$ by ANOVA with Bonferroni multiple comparisons test. P4pal-10 (purity > 95%) was synthesized by Biosyntan using the Fmoc solid-phase method, and quantified by amino acid analysis. Collagen was from Nycomed, AYPGKF and SFLLRN were from Bachem, and thrombin was a gift from J. Stürzebecher (Universität Jena).