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COMMENTARY

Proteinase-activated receptor pharmacology: trickier and trickier

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Proteinase-activated receptors (PARs) are G-protein-coupled receptors for serine and other proteinases. Peptide agonists of these receptors are frequently used to characterise the presence and role of PARs in cells and organ systems. However, the specificity of these peptides is questionable in some assay systems. In this issue, Hollenberg *et al*. report very different effects of PAR_4 receptors in various assays. Their results suggest the existence of unknown receptors and further highlight the need to use peptide PAR agonists with due caution.
British Journal of Pharmacology (2004) **143,** 441. doi:10.1038/sj.bjp.0705949

Keywords: Proteinase; proteinase-activated receptor; inflammation; coagulation

Abbreviations: PAR, proteinase-activated receptor

Proteinase-activated receptors (PARs) have attracted a great deal of interest, for a variety of reasons. Prominent among these is their unique mechanism of receptor activation: the N-termini contain the ligand that activates the receptors after being exposed subsequent to proteolytic removal of the more downstream sequence of amino acids (Hollenberg & Compton, 2002). Thus, these receptors essentially activate themselves *via* exposure of their tethered ligand. Since many of the proteinases that can activate PARs are involved in the coagulation cascade and its complex interaction with inflammatory processes, PARs are also interesting from a therapeutic point of view for the treatment of thrombotic and inflammatory diseases. An important pharmacological development in the field has been the synthesis of peptide-based agonists for PARs, based on the native sequence of the tethered ligand. However, the specificity of these peptides has been questioned several times (e.g. Saifeddine *et al*., 1998; Stenton *et al*., 2002). Similarly, proteinases used as agonists are rarely specific for a single PAR, or for PARs in general (Moffatt *et al*., 2004). At the moment, PARs are difficult to work with.

In this issue of the *British Journal of Pharmacology*, Morley Hollenberg's group – leaders in the study of structure–affinity relationships in this field – report very different PAR_4 agonist potencies in different bioassay systems. This finding suggests that in addition to PAR_4, there appear to be unknown receptors in other assay systems that are responsive to PAR_4-activating peptides. Whether they are responsive to proteinases remains to be seen. A novel, and more worrying, observation is that partially scrambled peptide sequences, which have often been used as control agonists (i.e. incapable of activating PAR_4), appear to exert biological effects in these assays. By contrast, in a platelet aggregation assay, a sensitive and reliable assay for PAR_4, these peptides are inactive. Finally, their data suggest that novel palmitoylated antagonists that inhibit PAR-G protein interactions may act as agonists in some assays. The implication is that PAR_4 pharmacology is likely to be quite confused outside the realms of isolated cells, such as platelets, that express abundant PAR_4 without the complication of unidentified receptor populations. Identifying these receptors will be an important task for the future. Furthermore, the agonist-like activity of palmitoylated antagonists, which are structurally unrelated to modified PAR tethered ligand sequences, suggests that this drug design strategy might have flaws.

In an era obsessed with reductionist molecular approaches to asking questions in the biomedical sciences, there is also something invigorating about seeing traditional pharmacological thinking and bioassays used to such clear effect. In the PAR field, we are fumbling in the dark as much as our predecessors were 50 years ago with receptors for which there are now numerous highly selective ligands. There are four PARs that we know of and now plenty of worrying evidence that there are unidentified receptors that are activated by currently utilised agonists (and even scrambled sequences) that are generally held to be PAR-specific. There are few reliable antagonists available and desensitisation experiments, where possible, have obvious pitfalls. The message is simple: the strength of future studies will depend on a dedicated pharmacological approach.

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Advance online publication: 20 September 2004

(Received June 22, 2004
Accepted July 9, 2004)