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COMMENTARY

Towards a Receptor for Nocistatin?

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Nocistatin is a peptide derived from the pronociceptin precursor, the source of nociceptin, the endogenous ligand for the nociceptin (NOP or ORL1). Despite nocistatin showing activity in a wide range of assays for nociception and other CNS activities, there is a dearth of information regarding the cellular actions of this peptide in the brain, and no receptor for nocistatin has been identified. In a study published in this issue of the *British Journal of Pharmacology*, Fantin and colleagues demonstrate that nocistatin inhibits 5-HT release from cortical synaptosomes in a concentration-dependent and *Pertussis* toxin-sensitive manner. The actions of nocistatin are independent of activity at NOP receptors. This study represents the first unambiguous demonstration of nocistatin agonist actions in brain and, taken together with previous work in the spinal cord, provides strong evidence that there is an as yet unidentified G protein-coupled receptor for nocistatin.
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Abbreviations: NOP, nociceptin receptor

Neuropeptide transmitter systems are fascinating for many reasons, not the least of which is that neuropeptides are usually encoded by a larger precursor that contains several active peptides. Precursors can contain structurally distinct ligands for related receptors, such as the various opioid receptor ligands found in the proenkephalin and prodynorphin molecules while other precursors consist of ligands for very different receptors, such as the opioid agonist β-endorphin and multiple melanocortin receptor agonists found in proopiomelanocortin. Nociceptin, the endogenous ligand for the opioid-related receptor (NOP), is also derived from a larger precursor that encodes several other peptides with central nervous system activity. The best characterized of these peptides is nocistatin, so named because it blocked the pro-nociceptive effects of nociceptin when the peptides were injected together into the spinal cord of mice (Okuda-Ashitaka *et al*., 1998). The original study describing the actions of nocistatin showed that it had no activity at recombinant NOP receptors and also reported the specific binding of a nocistatin analogue to brain membranes. The minimum active sequence of nocistatin was soon confirmed across several species and nocistatin was identified in human brain (Lee *et al*., 1999). Identification of a receptor for nocistatin seemed only a matter of time.

We are still waiting.

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Despite several dozen studies describing diverse biological actions of nocistatin, only small steps have been taken towards understanding the nature of its receptor. In the present issue of this Journal, Fantin and colleagues (Fantin *et al*., 2007) report another such step and, while it brings us no closer to the molecular identification of a nocistatin receptor, their results provide more strong evidence that a G protein-coupled receptor for nocistatin exists on neurons. In this study, Fantin *et al*. (2007) examined the effects of nocistatin on depolarization-evoked release of [^{3}H]5-HT from cortical synaptosomes. The most important finding of the study is simply that nocistatin inhibited the release of [^{3}H]5-HT in a concentration-dependent manner. They further showed that the effects of nocistatin did not depend on the expression or activity of NOP and they were blocked by loading the synaptosomes with *Pertussis* toxin, a selective inhibitor of signalling through G_i and G_o G proteins. To place these results into context, this study is the first to describe agonist actions of nocistatin in a system as simple as synapses or synaptosomes derived from brain. In an earlier series of landmark studies, Uli Zeilhofer and colleagues showed that nocistatin inhibited synaptic glycine and GABA release onto neurons in spinal cord slices and, as in the present study, they showed that the effects of nocistatin were abolished by *Pertussis* toxin and did not involve NOP receptors (Zeilhofer *et al*., 2000; Ahmadi *et al*., 2001, 2003).

The evidence for a nocistatin receptor seems compelling, so why do not we know more about it? A major problem has been the lack of tools to use to study the nocistatin-binding sites. The original radioligand used in the study of Okuda-Ashitaka *et al*. (1998) was not used again, and only very

recently has an alternative strategy of using biotinylated nocistatin to label binding sites been reported (Kazi *et al*., 2007). It may be that the nocistatin receptor has quite a low abundance and a study which reported the anatomical location of nociceptin-stimulated G-protein activation using GTPγS labelling failed to find any evidence for a similar signal from a nocistatin receptor (Neal *et al*., 2003). The few studies that have successfully demonstrated agonist actions of nocistatin have studied neurotransmitter release, which may imply a selective localization of the receptor in presynaptic elements, which are hard to access directly. Finally, it is possible that nocistatin does act at an already identified receptor, but does so in an unusual manner that is not readily detected in screening assays, for example by acting as an allosteric enhancer of agonist action.

Research into the effects of nocistatin has been largely defined by its relationship with nociceptin and this may also have hampered progress towards its emergence as a neurotransmitter in its own right. Many studies with nocistatin *in vivo* have primarily investigated how it affects nociceptin actions, including whether it blocks the inhibitory effects nociceptin has on μ-opioid analgesia, and *in vitro* studies have often focussed on whether nocistatin mimicked or blocked the effects of nociceptin, despite there being very limited evidence that the two peptides interact at a cellular level (Nicol *et al*., 1998). The study of Fantin *et al*. (2007) offers an intriguing hint that there may be interactions between the signalling pathways utilized by the nocistatin receptor and NOP receptors because pretreatment with a high concentration of nocistatin partially attenuated the inhibition of [^{3}H]5-HT release by a subsequent superfusion of nociceptin. In this experiment, the high concentration of nocistatin was by itself ineffective at inhibiting [^{3}H]5-HT release, perhaps because of receptor desensitization. The effect on the nociceptin response suggests that this desensitization is at least partly heterologous.

We hope this study will revive interest in nocistatin signalling in the brain and stimulate a renewed effort to find the nocistatin receptor. Perhaps 5HT neurons in the raphe complex are a good place to begin to look, as it was in these neurons that the first neuronal actions of nociceptin were described (Vaughan and Christie, 1996). Nocistatin has been shown to affect a number of complex behaviours, most notably those reflecting the processing of noxious stimuli. The possibility that drugs acting at a nocistatin receptor might be analgesic is just one good reason to find a receptor for this orphan agonist.

References

Ahmadi S, Kotalla C, Guhring H, Takeshima H, Pahl A, Zeilhofer HU (2001). Modulation of synaptic transmission by nociceptin/orphanin FQ and nocistatin in the spinal cord dorsal horn of mutant mice lacking the nociceptin/orphanin FQ receptor. *Mol Pharmacol* **59**: 612–618.

Ahmadi S, Muth-Selbach U, Lauterbach A, Lipfert P, Neuhuber WL, Zeilhofer HU (2003). Facilitation of spinal NMDA receptor currents by spillover of synaptically released glycine. *Science* **300**: 2094–2097.

Fantin M, Fischetti C, Trapella C, Morari M (2007). Nocistatin inhibits 5-hydroxytryptamine release in the mouse neocortex via presynaptic $G_{i/o}$ protein linked pathways. *Br J Pharmacol* **152**: 549–555 (this issue).

Kazi JA, Liu EH, Lee TL, Tachibana S (2007). Localization of nocistatin-binding sites in mice brain and spinal cord using a biotinylated nocistatin probe. *NeuroReport* **18**: 767–770.

Lee T-L, Fung FMY, Chen F-G, Chou N, Okuda-Ashitaka E, Ito S *et al*. (1999). Identification of human, rat and mouse nocistatin in brain and human cerebrospinal fluid. *NeuroReport* **10**: 1537–1541.

Neal Jr CR, Owens CE, Taylor LP, Hoversten MT, Akil H, Watson SJ (2003). Binding and GTPγS autoradiographic analysis of prepro-orphanin precursor peptide products at the ORL1 and opioid receptors. *J Chem Neuroanat* **25**: 233–247.

Nicol B, Lambert DG, Rowbotham DJ, Okuda-Ashitaka E, Ito S, Smart D *et al*. (1998). Nocistatin reverses nociceptin inhibition of glutamate release from rat brain slices. *Eur J Pharmacol* **356**: R1–R3.

Okuda-Ashitaka E, Minami T, Tachibana S, Yoshihara Y, Nishiuchi Y, Kimura T *et al*. (1998). Nocistatin, a peptide that blocks nociceptin action in pain transmission. *Nature* **392**: 286–289.

Vaughan CW, Christie MJ (1996). Increase by the ORL1 receptor (opioid receptor-like 1) ligand, nociceptin, of inwardly rectifying K conductance in dorsal raphe neurones. *Br J Pharmacol* **117**: 1609.

Zeilhofer HU, Selbach UM, Guhring H, Erb K, Ahmadi S (2000). Selective suppression of inhibitory synaptic transmission by nocistatin in the rat spinal cord dorsal horn. *J Neurosci* **20**: 4922–4929.