Home for an orphan endorphin

David Julius

OPIATES have maintained their place in history as both the most valued and most abused of pharmacological agents. Drugs such as morphine and heroin are as unparalleled in their capacity to relieve pain and induce euphoria as they are in their ability to enslave the user in a cycle of tolerance and addiction. The two primary breakthroughs in understanding the molecular basis of opiate action were the identification of specific opioid receptors in the central nervous system, and the purification of endogenous morphine-like substances (endorphins) from the brain[1]. On page 532 of this issue[2], Meunier *et al.* describe the isolation of a new seventeen-amino-acid-long peptide from rat brain that may be an endogenous agonist for an orphan member of the opioid receptor family.

Sequences of members of the endorphin family

Leu-enkephalin	Tyr-Gly-Gly-Phe-Leu-OH
Met-enkephalin	Tyr-Gly-Gly-Phe-Met-OH
β-Endorphin	Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Val-His-Lys-Lys-Gly-Gln-OH
α-Neoendorphin	Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro-Lys
Dynorphin	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln-OH
Nociceptin	Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln-OH

Tentatively dubbed 'nociceptin' for its ability to induce hyper-reactivity in response to a noxious thermal stimulus, this peptide shares greatest sequence similarity with dynorphin A, one of five established endogenous ligands for opioid receptors (see table). These receptors have been classified into three main subtypes (μ, κ and δ) and cloning efforts have shown that they belong to a closely related subfamily of G-protein-coupled receptors[3]. Using homology-based screening strategies, several groups independently identified a novel member of the family that does not bind any of the known opiate ligands with high affinity (see ref. 2 and references therein). It was therefore proposed that the endogenous ligand for this orphan receptor (termed ORL_1 for opioid receptor-like 1)[4] might be a novel opiate-related peptide[5].

Working from the knowledge that all three opioid receptor subtypes are coupled negatively to adenylyl cyclase, Meunier and co-workers found that very high concentrations of the opiate etorphine can inhibit adenylyl cyclase in transfected cells expressing ORL_1 receptors[4]. Starting with crude rat brain extracts, the authors used this assay to follow ORL_1 agonist activity through a series of biochemical fractionation steps, yielding a heptadecapeptide with nanomolar potency at this site.

All five of the major opioid peptides characterized so far bear the amino-terminal signature sequence Tyr-Gly-Gly-Phe[1,6]. Just as one might have hoped, the newly isolated peptide begins with the sequence Phe-Gly-Gly-Phe- and bears further resemblance to the opioid peptide dynorphin A. As is often the case for small peptide hormones and transmitters[7], the novel peptide appears to be synthesized as part of a larger polyprotein precursor. Thus, whenever such a precursor is found, there is the exciting possibility that additional peptide transmitters may be liberated by proteolytic cleavage at dibasic recognition sites that commonly flank the sequences of mature peptide hormones. If, as the authors suggest, this precursor polypeptide resembles pro-opiomelanocortin (from which β-endorphin and a variety of non-opioid peptides are derived), then they may have stumbled upon a treasure trove of new bioactive peptides.

A search of the gene bank database points out some additional bits of homology between the newly cloned precursor and other opioid genes within the presumptive pro-domain. This observation suggests that opioid ligands and their receptors may have evolved in parallel, maintaining some specific, high-affinity interactions along the way. Indeed, the novel peptide has no significant agonist activity at μ-, δ- or κ-opioid receptors[2], and broad-spectrum opioid antagonists such as naloxone have no appreciable affinity for ORL_1 (ref. 4). How, then, should we view ORL_1 and its novel ligand — as functionally unique members of the opioid family, or as the first members of an entirely new peptide signalling system?

Meunier *et al.* present data to suggest that intraventricular injection of synthetic peptide into the mouse brain increases an animal's reactivity to pain, in curious contrast to the analgesic actions of most opiate drugs. In the absence of specific antagonists, the authors must rely on antisense technology to corroborate these findings; they show that a reduction in ORL_1 receptor expression has the predicted opposite effect of decreasing the animal's reactivity to noxious stimuli. Assuming that receptor levels are, indeed, reduced in mice treated with antisense oligonucleotides, these findings would support a role for the ORL_1 receptor and the novel peptide in a nociceptive pathway.

Of course, hyper-reactivity can reflect changes in systems other than those directly related to nociception. So before such a role for this peptide can be fully accepted, it will be necessary to define the sites of peptide biosynthesis and action within the pain modulation pathway using anatomical and physiological techniques such as radioligand binding assays, immunohistochemical and *in situ* hybridization methods, and stereotactic microinjection of agonists and antagonists into discrete regions of the brain and spinal cord. For now, it can at least be said that ORL_1 receptor transcripts are expressed in several areas of the central nervous system that are known to be involved in pain regulation, including the hypothalamus, brainstem and spinal cord dorsal horn[4,5,8].

Given the powerful euphoric and addictive properties of opiate drugs, it is important to consider the actions of opioid ligands in limbic system function and plasticity. ORL_1 receptor transcripts are, in fact, highly expressed in limbic system regions such as the cerebral cortex, amygdala and hippocampus[4,5,8]. This may be interesting in light of findings that dynorphin can act as a neurotransmitter at glutamatergic mossy fibre synapses within the hippocampus[9]. By activating κ-opioid receptors in the mossy fibre pathway, dynorphin can depress synaptic transmission and inhibit the induction and expression of long-term potentiation. Nociceptin, acting at ORL_1 receptors, may play a similar role in modulating synaptic plasticity in the central nervous system. □

David Julius is in the Department of Cellular and Molecular Pharmacology, Box 0450, University of California, San Francisco, California 94143, USA.

1. Snyder, S. H. *Drugs and the Brain* (Freeman, New York, 1986).
2. Meunier, J.-C. *et al. Nature* **377,** 532–535 (1995).
3. Reisine, T. *Neuropharmacology* **34**, 463–472 (1995).
4. Mollereau, C. *FEBS Lett.* **341**, 33–38 (1994).
5. Lachowicz, J. E., Shen, Y., Monsma, F. J. & Sibley, D. R. *J. Neurochem.* **64**, 34–40 (1995).
6. Fields, H. L. *Pain* (McGraw-Hill, New York, 1987).
7. Herbert, E. & Uhler, M. *Cell* **30**, 1–2 (1982).
8. Bunzow, J. R. *et al. FEBS Lett.* **347**, 284–288 (1994).
9. Weisskopf, M. G., Zalutsky, R. A. & Nicoll, R. A. *Nature* **362**, 423–427 (1993).