Neuropharmacology

Another opiate for the masses?

David Julius

The active ingredients of natural toxins and herbal remedies have helped to unlock the secrets of biological processes ranging from cell division to synaptic transmission. A notable example is opium — an extract of the poppy plant that has been used for centuries to induce euphoria and relieve pain. The active component of opium is morphine, and its synthetic congener is heroin[1,2]. The analgesia, reward-seeking behaviour and physical dependence elicited by morphine[2,3] are mainly mediated through the μ opioid receptor and, on page 499 of this issue, Zadina and colleagues[4] report the isolation of two new μ-selective peptide ligands from mammalian brain. These peptides (called endomorphin-1 and -2) rival morphine in both their potency as agonists and their analgesic activity. If they are, indeed, natural ligands for the μ receptor, then they should provide new molecular tools for dissecting the endogenous opiate pathways in the brain and spinal cord.

The quest to determine how the opiate alkaloids mediate euphoric, analgesic and addictive states has helped to shape a central tenet of modern neuropharmacology: namely, that toxins and drugs usurp sites at which endogenous factors normally act to transduce biological signals. Two discoveries affirmed this hypothesis. First, radiolabelled opiate alkaloids were shown to bind to specific, high-affinity sites in the brain, corresponding to three distinct guanine-nucleotide-binding (G) protein-coupled opioid-receptor subtypes, termed κ, μ and δ[5] (Fig. 1). Second, *end*ogenous m*orphine*-like substances were identified in the brain. These so-called endorphins are peptides that activate opioid-receptor subtypes, with characteristic rank-order potencies. But, until now, no single endorphin had been shown to bind with both high selectivity and high affinity to the μ receptor, begging the question of whether the true 'endogenous morphine' had, in fact, been discovered.

The route that was taken by Zadina *et al.*[4] to uncover the new endomorphins is interesting in itself, because it involved an unusual interplay between pharmacology and combinatorial chemistry. The story begins with the isolation of a tetrapeptide called Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2) from bovine and human brain[6]. This peptide acts as both an agonist and an antagonist in physiological assays for binding to the μ receptor, which measure the opiate-dependent inhibition of electrically induced contractions in the guinea-pig ileum. An antibody was generated to Tyr-MIF-1, then a radioimmunoassay was used to isolate a related

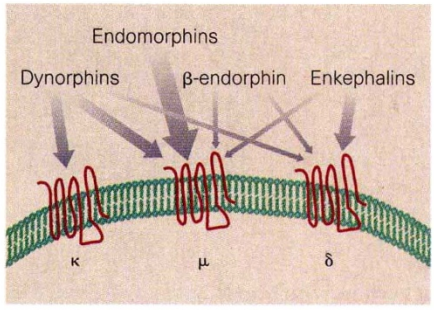

Figure 1 **Known agonists for the κ, μ and δ opioid receptors. Zadina *et al.*[4] have identified two naturally occurring endorphins (termed endomorphin-1 and -2) which bind with high specificity and high affinity to the μ receptor. Relative affinities of various agonists for different opioid-receptor subtypes are depicted by arrows.**

peptide from mammalian brain extracts[7], Tyr-W-MIF-1 (Tyr-Pro-Trp-Gly-NH_2). Tyr-W-MIF-1 is a more potent opiate agonist than Tyr-MIF-1, and it has a respectable selectivity for μ receptors over δ or κ receptors (200–300-fold). But its affinity for μ receptors (based on competitive binding assays with the μ-selective endorphin analogue ^{3}H-DAMGO) is modest (K_i 70 nM).

To identify a peptide with greater affinity for the μ receptor, Zadina *et al.* used a solid-phase synthesis technique known as polyethylene pin technology to generate 20 Tyr-W-MIF-1 derivatives, representing all of the possible amino-acid substitutions at the fourth position of the peptide. Phe4 — the peptide with phenylalanine at the fourth site (Tyr-Pro-Trp-Phe-NH_2) — turned out to have the desired characteristics, including sub-nanomolar affinity for μ receptors, and very high selectivity of binding to μ versus δ (>4,000-fold) or κ (>15,000-fold) sites. When it was injected into the brain or spinal column of mice, synthetic Phe4 proved to be as potent as morphine in producing a long-lasting analgesic response. Moreover, the response was reversed by administration of broad-spectrum or μ-selective opiate antagonists.

Next, the authors took a daring leap of faith — they predicted that the nervous system might express its own version of the Phe4 peptide. Using an antibody that was specific for the Phe4 peptide, they isolated immunologically crossreactive material from bovine brain extracts. These extracts indeed contained endogenous Phe4 peptide (endomorphin-1), along with the closely related species Tyr-Pro-Phe-Phe-NH_2 (endomorphin-2). Because other endorphins contain the amino-terminal signature sequence Tyr-Gly-Gly-Phe, it may be that a stable structure imposed by the proline residue at the second position helps to increase selectivity for the μ receptor. Furthermore, the long-lasting analgesic activity of endomorphin-1 and -2 could reflect protection from exoproteolytic degradation conferred by the carboxy-terminal amidation.

By analogy with other peptide hormones and neurotransmitters[8], including the endorphins, one would expect the endomorphins to be excised from a larger polyprotein precursor. Cloning the gene for this hypothetical pro-endomorphin would serve at least three purposes. First, it would confirm that the endomorphins are indeed endogenous neuropeptides, derived by specific proteolytic cleavage events. Second, sequence analysis of the gene would clarify any genetic relationship between endomorphins and the peptides that led to their isolation, including Tyr-MIF-1 and Tyr-W-MIF-1, and other biologically active peptides might be discovered in the process. Third, the endomorphin gene would provide sensitive and specific molecular probes for determining where these peptides are expressed in the nervous system. If the endomorphins are the endogenous ligands for μ receptors, they might be found in pain-modulating, reward-seeking or emetic centres of the nervous system (for example, the spinal-cord dorsal horn, periaqueductal grey, area postrema, striatum, amygdala and nucleus accumbens)[2]. Nonetheless, questions about the peptide distribution can perhaps be addressed with the specific antibody that was used by Zadina and colleagues[4] to purify the endomorphins.

Could the endomorphins provide relief from pain without eliciting the negative symptoms that are associated with morphine, such as respiratory depression, nausea, tolerance and addiction? Although pharmacological and genetic studies[3] indicate that this is unlikely, the answer may be 'yes' if the endomorphins are more μ-selective than morphine, or if the activation of μ receptors by structurally distinct agonists has differential effects on signalling. We next need to examine interactions between the endomorphins and cell lines that express cloned opioid receptors. Finally, does this work signal the beginning or the end of the search for endogenous opiate peptides? The modest binding selectivity of other known endorphins to the three opioid-receptor subtypes indicates that peptides with comparable selectivity for the δ and κ receptors are out there waiting to be discovered. □

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1. Snyder, S. *Drugs and the Brain* (Freeman, New York, 1986).
2. Fields, H. *Pain* (McGraw-Hill, New York, 1987).
3. Matthes, H. *et al. Nature* **383,** 819–823 (1996).
4. Zadina, J. E., Hackler, L., Ge, L.-J. & Kastin, A. J. *Nature* **386,** 499–502 (1997).
5. Kieffer, B. *Cell. Mol. Neurobiol.* **15,** 615–635 (1995).
6. Horvath, A. & Kastin, A. *J. Biol. Chem.* **264,** 2175–2179 (1989).
7. Erchegyi, J., Kastin, A. & Zadina, J. *Peptides* **13,** 623–631 (1992).
8. Herbert, E. & Uhler, M. *Cell* **30,** 1–2 (1982).