The distribution of methionine-enkephalin and leucine-enkephalin in the brain and peripheral tissues

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Commentary by

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This paper demonstrated a rapid method for extracting and quantitating opioid peptides from the brain and other tissues using the mouse vas deferens bioassay. It was established by chromatographic methods that over 90% of the total bioassable activity in brain was due to enkephalin-like peptides and the variation in the ratio of (met^5)- to (leu^5)-enkephalin varied from near unity to more than 8:1 in some brain areas. It was also demonstrated for the first time that there were substantial amounts of enkephalin-like materials in the gastrointestinal tract and that other peripheral organs including the heart and sympathetic nerves contained detectable enkephalin-like activity.

No one would now consider using bioassay as a means of measuring opioid peptide activity but Terry Smith and I wished to answer certain critical questions posed by the discovery of the enkephalins (Hughes *et al.*, 1975) and endorphins (Li & Chung 1976). These questions could not readily be answered by radioimmunoassay even if we had possessed the necessary antisera which we did not at that early stage in the field. Roger Guillemin (Guillemin, Ling & Burgus, 1976) had raised the possibility that the enkephalins were artifacts arising from the breakdown of the larger endorphins during either extraction or purification. It was important to show that varying the conditions of extraction did not significantly influence the bioassayable content of the tissues. Terry and I found that brain exsanguination and formalin fixation had no effect on the content of enkephalin although there was a significant loss of bioassayable activity if the brains were left *in situ* for one hour prior to extraction, a result hardly consistent with the artificial generation of enkephalins. We used the important control of the pituitary gland to show that our methods were equally good at detecting the larger opioid peptides although in retrospect we should also have tested the effect of formaline infusion on pituitary opioid content which we did not because of the difficulties in assaying such small amounts of material.

At this stage of development of the field there was no information as to the origin of the enkephalins although Howard Morris (Hughes *et al.*, 1975) had noted the presence of the (met^5)-enkaphalin sequence in the carboxy terminal sequence of β-lipotropin (subsequently named β-endorphin). It was to be some years before the identification of the precursor molecules pre-proenkephalin (PPE) and pre-prodynorphin (PPD). Thus the variation which we noted in the ratio of the two enkephalins in different brain areas was accounted for by the fact that (leu^5)-enkephalin could be generated from both PPE and PPD and thus variations in the distribution of the precursors influenced the ratio of enkephalins.

The discovery of enkephalin-like activity in the gut was not too surprising given the emerging concept of brain-gut peptides arising from a common neuroectodermal origin but the finding neatly tied together the correlation between peripheral and central opioid receptors. The presence of enkephalin in the gastrointestinal tract and other peripheral tissues was subsequently confirmed by the elegant immunohistochemical techniques of Tomas Hökfelt and Bob Elde. The role of enkephalins in the peripheral tissues remains a relatively neglected field apart from the gut. Hökfelt and his colleagues went on to establish the co-existence of the enkephalins (and other neuropeptides) in nerves with other neurotransmitters such as noradrenaline, acetylcholine and 5-hydroxytryptamine thus demolishing the dogma (wrongly attributed to Henry Dale) of "one nerve one transmitter".

In those early days of "neuropeptide excite-

ment" we were eager to try and ascribe function to these new messengers which included substance-P, neurotensin and the hypothalamic-pituitary peptides. The idea that the opioid peptides were something special and could give us a special insight into pain, addiction and other modalities was seductive. Indeed the opioid peptides have emerged as complex and widely-represented systems in the nervous system but there are many other neuropeptides with as great a claim to fame including somatostatin, galanin, neuropeptide-Y and many others. Why were opioids special? The answer may be rooted in the pharmacology of the opioid system, it is still the only peptide system to have both non-peptide agonist and antagonist tools with a defined pharmacology and clearcut therapeutic utilities. Indeed one enduring mystery is why "nature" did not produce agonists or antagonists to other neuropeptides, the absence of evolutionary pressure to select such entities may argue against a central role for any one neuropeptide system. Most recently this view was supported by the finding that deletion of the mu-opioid receptor in mouse stem cells lead to the production of mouse strains with no mu-opioid responsiveness yet the mice are apparently normal in relation to pain sensitivity and other behavioural characteristics (Matthes *et al.*, 1996). Nevertheless the endorphin, the enkephalin and the dynorphin peptides and their receptors are subject to complex and exquisitely fine controls and this and the constancy of the systems across species argues for important physiological roles for these peptides and their receptors. Indeed mice in which the enkephalin precursor gene is deleted showed increased pain sensitivity in some tests, aggressive behaviour and decreased exploratory activity (Konig *et al.*, 1996).

The opiates have long been associated with human societies and their sub-cultures. It is a sobering fact that today our therapeutic armamentarium for the treatment of pain still largely rests on the pharmacology of morphine and that opiate abuse still represents a major threat to our Society. The translation of basic research into practical applications is frustratingly slow but I am convinced it will eventually come to fruition in the opioid field. There is presently progress in developing new analgesics based on the discovery of the delta- and kappa-opioid receptors and inhibitors of enkephalin breakdown.

Hans Kosterlitz and I resisted the temptation to ascribe function to the enkephalins by adopting the American name endorphin and we followed physiological precedent by naming the peptides after their site of discovery in the "head". In the event there were more than enough endogenous opioid peptides to satisfy everyone's terminology but it is a source of some pleasure that the enkephalins proved to be the most abundant and widely distributed of the opioid peptides and not just artifacts!

Terry Smith eventually left Aberdeen to work at the Wellcome Research Laboratories in Beckenham and later joined the National Research and Development corporation. We still maintain contact and remain united in our appreciation of Hans Kosterlitz and his contribution to pharmacology.

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