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NEUROTROPHIC EFFECTS OF BETA ENDORPHIN C-TERMINAL TETRAPEPTIDE (MPF)

J.S. Morley[1] and D.M. Ensor[2]

[1]Pain Relief Foundation, Walton Hospital, Rice Lane, Liverpool L9 1AE, UK
[2]Dept. of Zoology, University of Liverpool, P.O. Box 147, Liverpool L69 3BX
(Reprint requests to JSM)

ABSTRACT

The ability of newts and other urolele amphibians to regenerate an accidentally-removed or amputated limb is lost after hypophysectomy, but restored following intraperitoneal injection of beta endorphin, or the C-terminal tetrapeptide sequence of human beta endorphin, Lys-Lys-Gly-Glu (=MPF). The closely related tetrapeptide, Lys-Lys-Gly-Gln (the C-terminal sequence of pig, sheep, and camel beta endorphin), the dipeptide Gly-Glu, the enkephalins, and other N-terminal sequences of beta endorphin (eg gamma endorphin) do not elicit the effect.

INTRODUCTION

Since the discovery of the existence of peptides in neurons, research on the role of neuropeptides has been dominated by attempts to establish their involvement in neurotransmission. We have argued, however, that there may be other equally important roles that can loosely be described as trophic (1). There is already evidence of such roles in the case of vasopressin, bombesin, substance P, and substance K (2). We now confirm an earlier report (3) that beta endorphin has an important role in the initiation of forelimb regeneration in the adult newt, and show that this role is associated with part of the beta endorphin molecule, ie the C-terminal tetrapeptide sequence Lys-Lys-Gly-Glu (=MPF).

MATERIALS AND METHODS

Adult italian (Triturus cristatus), or chinese (Cynops cymurus), newts, supplied, respectively, by Griffin & George, Bristol, and Bioserv Limited, Somerset, England, were anaesthetised by immersion in 1% aqueous urethane. Each was then placed on their back between two moist layers of blotting paper, and gently secured with a rubber band, prior to hypophysectomy by means of a small dental drill. The success of hypophysectomy was later checked by histological examination. The distal part of one forelimb was removed just below the elbow joint with scissors, and the open wound was sutured. In each of two experiments, groups of five newts were segregated at random and kept in aquaria. Test compounds were freshly dissolved in saline, stored at 4°C, and administered intraperitoneally 1, 4, 7, and 10 days after amputation. Blastema growth was measured 21 days after amputation by means of a micrometer guage. Beta

endorphin, gamma endorphin, and ∝-MSH were purchased from Cambridge Research Biochemicals, Cambridge, England. The C-terminal tetra peptide sequence of human beta endorphin, the dipeptide Gly-Glu, and Lys-Lys-Gly-Gln were prepared by classical methods of peptide synthesis. The purity of all samples was assessed as >95% by thin layer chromatography, high performance liquid chromatography, and/or paper electrophoresis, and amino acid analysis of acid and/or enzymic digests.

RESULTS

Normal amputated newts, and normal amputated newts receiving saline had well-formed cone regenerates at 21 days after amputation, whereas hypophysectomised newts, and hypophysectomised newts receiving saline failed to form even an accumulating bud. Hypophysectomised newts receiving beta endorphin (10 ug per injection), Lys-Lys-Gly-Glu (MPF)(100 ug per injection), or ∝-MSH (100 ug per injection) failed to regenerate. Where regeneration occurred, the amount of regeneration was not significantly different from that seen in the controls (Tables 1 & 2). The dose of 100 ug per injection for all compounds except beta endorphin was chosen arbitrarily, and the effect of lower doses has not yet been investigated.

Table 1. Forelimb regeneration (Triturus cristatus)

Peptide	Intraperitoneal dose (ug)	Mean growth (mm.)±SE	(n)
Beta endorphin	10	1.06 ± 0.09	(5)
Gamma endorphin	100	0.11 ± 0.02	(5)
Lys-Lys-Gly-Glu (MPF)	100	1.06 ± 0.08	(5)
Control (intact)	-	1.72 ± 0.10	(5)
Control (hyp/ect)	-	0.01 ± 0.01	(5)

Control (intact) newts had a forelimb only removed; Control (hyp/ect) newts had a forelimb and the hypophysis removed.

Table 2. Forelimb regeneration (Cynops cymurus)

Peptide	Intraperitoneal dose (ug)	Mean growth (mm) ± SE	(n)	Significance v. Control (hyp/ect)
Beta endorphin	10	1.20 ± 0.09	(5)	$p < 0.001$
Lys-Lys-Gly-Glu (MPF)	100	1.12 ± 0.01	(5)	$p < 0.001$
Lys-Lys-Gly-Gln	100	0.18 ± 0.01	(5)	N.S.
Gly-Glu	100	0.20 ± 0.03	(5)	N.S.
∝-MSH	100	1.24 ± 0.02	(5)	$p < 0.001$
Control (intact)	-	1.86 ± 0.09	(5)	
Control (hyp/ect)	-	0.14 ± 0.008	(5)	

Controls as Table 1. N.S. = not significant.

DISCUSSION

It has long been known that the presence of intact nerves is essential during the early stages of limb regeneration in newts, corresponding to the blastema accumulation and proliferation stages (4). The precise role of neural input is not known, but evidence supports a role in which neuronal messengers influence the decision of blastemal cells either to proliferate, or to prepare to express differentiated phenotypes (5). Our results suggest that beta endorphin, and more specifically its C-terminal tetrapeptide sequence, is at least one of these messengers.

Of particular interest is the finding that this role of beta endorphin is related to the C-terminal and not the N-terminal regions of the molecule - the C-terminal tetrapeptide, Lys-Lys-Gly-Glu (MPF), was as effective as human beta endorphin in eliciting the regeneration, whereas two N-terminal fragments, [Met]enkephalin (1-5) and gamma endorphin (1-17), were ineffective. It is therefore highly unlikely that the effect is mediated via an opioid receptor.

There is, indeed, growing evidence of important non-opioid actions of opioid peptides mediated by receptors with high affinity for C-terminal regions of the beta endorphin molecule. An early study showed that the C-terminal tetrapeptide markedly potentiated the action of melanotrophin in steady state or rate assays of the peptide - hence description of the tetrapeptide as melanotrophin potentiating factor (MPF) (6). More recently, human beta endorphin, but not gamma endorphin (1-17), has been shown to modulate T-cell mitogen-induced proliferation of T lymphocytes, and the effect could not be blocked by naloxone (7,8). Specific binding to the terminal complexes of human complement is seen with human beta endorphin and MPF, but not at all with alpha endorphin (1-16), endorphin 1-27, or camel beta endorphin (which has Gln in place of C-terminal Glu) (9). At the biochemical level, camel beta endorphin, and the C-terminal dipeptide of pig, sheep, and camel beta endorphin, i.e. Gly-Gln, regulate the formation of A_{12} acetylcholinesterase in cultured embryonic muscle cells (10), and may have important roles in maintaining synaptic levels of acetyl choline in developing neuromuscular junctions (11).

Our preliminary results do not permit full definition of the structural requirements at the C-terminus of human beta endorphin for elicitation of the forelimb regeneration effect (further studies are in progress). Clearly, however, structural specificity is high, since amide formation at the gamma carboxyl group of the C-terminal glutamic acid residue (change of $-NH-CH(CH_2CO_2H)-CO_2H$ to $-NH-CH(CH_2CONH_2)-CO_2H$) provided an analogue that did not give rise to the effect (Table 2; result with Lys-Lys-Gly-Gln). By implication, it may be predicted that pig, sheep, and camel beta endorphins will also be inactive, but this prediction has not yet been tested. A further implication is that the beta endorphin of urodele amphibians, like that of the human species, contains C-terminal glutamic acid. If the structural requirements for the initiation of forelimb regeneration are similar to those for melanotrophin potentiation, the 'minimal fragment' of human beta endorphin required for the effect will be the C-terminal tetrapeptide sequence (MPF). The C-terminal tripeptide, Lys-Gly-Glu, has not yet been examined, but, in agreement, the C-terminal dipeptide, Gly-Glu, was inactive (Table 2).

On the basis of our results, we speculate that the C-terminal tetrapeptide (MPF), arising from intraneuronal processing of beta endorphin, or by

or by independent processing of proopiomelanocortin, may be the molecular species delivered by nerves to the damaged tissue after amputation. Stress is known to cause increased proopiomelanocortin synthesis, reflected in several-fold increases in plasma concentrations of both ACTH and beta endorphin (12). However, plasma levels of beta endorphin increase much less than those of ACTH (13,14). So, if we assume that plasma levels are indicative of neuronal activity, it could be that the deficiency in beta endorphin is reflected in increased levels of MPF (which is not detected by common radioimmunoassay methods for measuring beta endorphin). In other words, stress may be related to altered processing of proopiomelanocortin, providing a higher proportion of MPF-like peptides. It may be argued that useful levels of MPF are unlikely to arise at nerve terminals because the presence of two Lys residues in the molecule render it susceptible to tryptic degradation. In fact, the stability of MPF towards trypsin is high (15). Furthermore, we have speculated that many of the events associated with peptides in neurons arise via non-synaptic contacts with other cells (1), and the nature of proteases within this environment is unknown.

It will be noted that α-MSH is also able to cause forelimb regeneration in hypophysectomised newts (Table 2). Further studies are in progress to determine if the the regeneration by α-MSH is qualitatively similar to that provoked by MPF.

The possibility arises that MPF may also have trophic significance in the mammalian nervous system. This possibility is being explored using nerve cultures.

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