ADVANCES IN PHARMACOLOGY AND THERAPEUTICS

Proceedings of the 7th International Congress of Pharmacology

General Editors: J. R. BOISSIER, P. LECHAT & J. FICHELLE

Volume 1

RECEPTORS

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Introduction

The scientific contributions at the 7th International Congress of Pharmacology were of considerable merit. Apart from the sessions organised in advance, more than 2,200 papers were presented, either verbally or in the form of posters, and the abundance of the latter in the congress hall is a good indication that this particular medium of communication is becoming increasingly attractive to research workers, and offers scope for discussions which combine an elaborate, thorough approach with a certain informality.

It would have been preferable to have published the entire congress proceedings within the framework of the reports. That was, however, physically impossible, and the organisers had to adopt a realistic solution by publishing only the main lectures, symposia and methodological seminars. The amount of material presented necessitated the printing of ten volumes, each volume containing congress topics regrouped according to their relevant content and subject areas. This system of division may give rise to criticism on account of its artificiality, and we readily admit that certain texts could have been placed in more than one volume. We are asking the reader to excuse this arbitrariness, which is due to the editors' personal points of view.

I draw attention to the fact that most of the symposia finish with a commentary which the chairmen had the option of including, presenting their personal opinions on one or several points. We think that such an addition will facilitate reflection, discussion, indeed even controversy.

The launching of the scientific programme for this congress began in September 1975 on returning from the last meeting in Helsinki. Long and delicate discussions took place in the Scientific Programme Committee and with the International Advisory Board. Should it be a pioneer, 'avant-garde' congress? Or one laid out like a balance-sheet? Should we restrict the congress to the traditional bounds of pharmacology, or extend the range of papers to cover the finest discipline? The choice was difficult, and the result has been a blend of the two, which each participant will have appreciated in terms of his training, his tastes, and his own research.

A certain number of options, however, were taken deliberately: wide scope was given to toxicology, from different points of view, and to clinical pharmacology, a subject much discussed yet so badly practised; the founding of two symposia devoted

to chemotherapy of parasitic diseases which are still plagues and scourges in certain parts of the world; a modest but firm overture in the field of immunopharmacology, which up until now was something of a poor relation reserved only for clinical physicians; the extension of methodological seminars, in view of the fact that new techniques are indispensable to the development of a discipline.

We have been aware since the beginning that, out of over 4,000 participants who made the journey to Paris, not one could assimilate such a huge body of knowledge. Our wish is that the reading of these reports will allow all of them to become aware of the fantastic evolution of pharmacology in the course of these latter years. If one considers pharmacology as the study of the interactions between a "substance" and a living organism, then there is no other interpretation. Nevertheless, one must admit that there exists a period for describing and analysing a pharmacological effect, and that it is only afterwards that the working mechanism can be specified; a mechanism which will permit these "substances" to be used for the dismantling and breaking down of physiological mechanisms, a process which justifies Claude BERNARD'S term, "chemical scalpel".

The reader will be able to profit equally from more down-to-earth contributions, more applied to therapeutics, and less "noble", perhaps, for the research worker. He will realise then that his work, his research and his creative genius are first and foremost in the service of Man, and will remember this statement from Louis PASTEUR:

> "Let us not share the opinion of these narrow minds who scorn everything in science which does not have an immediate application, but let us not neglect the practical consequences of discovery."

I would like to renew my thanks to my colleagues in the Scientific Programme Committee and also to the members of the International Advisory Board, whose advice has been invaluable. I owe a particular thought to J J BURNS, now the past-president of IUPHAR, who granted me a support which is never discussed, and a staunch, sincere friendship. The Chairmen have effected an admirable achievement in the organisation of their proceedings, and in making a difficult choice from the most qualified speakers. The latter equally deserve our gratitude for having presented papers of such high quality, and for having submitted their manuscripts in good time.

The publisher, Robert MAXWELL, has, as always, put his kindness and efficiency at our service in order to carry out the publication of these reports. But none of it would have been possible without the work and competence of Miss IVIMY, whom I would like to thank personally.

My thanks again to the editors of the volumes who, in the middle of the holiday period, did not hesitate to work on the manuscripts in order to keep to the completion date.

Finally, a big thank you to all my collaborators, research workers, technicians and secretaries who have put their whole hearts into the service of pharmacology. They have contributed to the realisation of our hopes for this 7th International Congress, the great festival of Pharmacology. Make an appointment for the next one, in 1981, in Tokyo.

Jacques R BOISSIER

Chairman

Scientific Programme Committee

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Chemistry and Biochemistry of Pituitary Endorphins

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ABSTRACT

Correlation has been demonstrated between the analgesic potency, receptor binding properties, preferred solution conformation and metabolic stability of natural and synthetic opioid peptides. Of several factors contributing to the analgesic effect, preference has been given to the improved receptor binding affinity and specificity provided by either a putative hydrophobic bonding between the C- and N-terminal parts of β-endorphin, or the occurrence of Pro at the C-terminus of some superactive enkephalin analogs. Progress has been made in the isolation and characterization of a particle-bound pituitary endopeptidase involved in the generation of β-endorphin from β-lipotropic hormone.

INTRODUCTION

Stimulated by the discovery of brain enkephalins /1/ and their structural relatedness to β-lipotropic hormone /β-LPH/, a 91-residue polypeptide of pituitary origin /2,3/, a number of opioid peptides have been isolated from the pituitary gland /for reviews see Refs 4,5/. These peptides designated as endorphins, have been shown to be β-LPH fragments of different length having in common the Met-enkephalin structure at their N-terminus /Fig. 1/. Though immunocytochemical and radioimmunassay studies have provided ample evidence for the occurrence of β-LPH- and β-endorphin-like polypeptides in different brain regions also /6,7/, these substances have not been isolated and chemically identified yet. This paper is confined to pituitary endorphins only, and attempts to review some of the major achievements of the last three years regarding the structural basis of the morphine-like activity of endorphins and also the mechanism of their generation in the pituitary gland.

STRUCTURAL REQUIREMENTS FOR ANALGESIC EFFECT

There have been two main classes of assays to explore structure-function relationships in substances with morphine-like properties:

in vitro test systems /guinea pig ileum, mouse vas deferens and receptor binding assay/ and in vivo assays for analgesic potency /tail flick test, hot plate test, etc./.

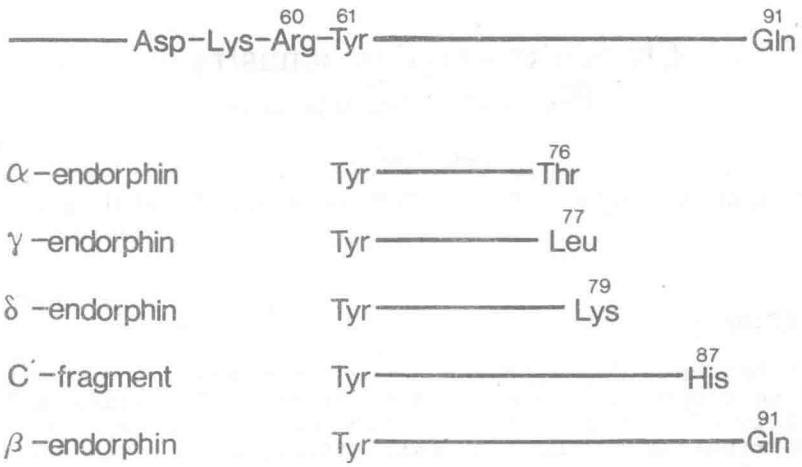

Fig. 1. Schematic representation of the structural relationships among β-LPH and endorphins

Utilizing in vitro assays, extensive structure-activity data have been available to evidence that the presence and relative position of the aromatic residues, Tyr^{61} and Phe^{64}*, in the enkephalin structure are quintessential for the biological response /for review see Ref. 8/: Thus, Tyr^{61} and Phe^{64} would be directly involved in the 'activation' of the opiate receptor, whereas the Gly residues at positions 62 and 63 may serve as spacers to hold the aromatic side chains in proper positions. The tetrapeptide derived by the removal of the C-terminal Met or Leu from enkephalins retains some affinity to brain opiate receptors /9/ and has full intrinsic activity in the guinea pig ileum bioassay /4/ implying that the C-terminal residue in enkephalins may represent an additional binding site of the molecule. It has also been speculated that the same residue contributes to a hydrogen-bonded conformation adopted by the enkephalin molecule upon interaction with the receptor /10,11/. The high biological potency of enkephalin analogs with Pro as C-terminus /12,13/ however, indicates that a β-turn comprising residues 62-65 cannot be exclusive for opiate activity. A previous proposal of β-turn for residues 61-64 /14/ rather than 62-65 has been supported by a more recent study on the conformational similarities of Met-enkephalin to rigid opiates /15/.

*All the residue numbers used correspond to the β-LPH structure /3/.

Contrary to the initial expectations, enkephalins failed to exert significant analgesic activity by central administration /16,17/. Subsequent structure-activity studies on pituitary endorphins have revealed that analgesic activity is a more or less unique property of β-endorphin /17,18, Table 1/. As it appears from the comparison of the bioassay data in Table 1, β-endorphin is distinguished from the shorter opioid peptides not only by its in vivo effect but also by its in vitro activities /20,21/. The relatively high guinea pig ileum/mouse vas deferens potency ratio obtained for β-endorphin /20,21, Table 1/ together with its increased binding affinity to the brain receptors /23/ suggests that the extreme analgesic activity may primarily be accounted for by a unique structural feature of the molecule to produce preferential and specific interaction with some opiate receptors in the brain. The differential behaviour of β-endorphin in different model systems is clearly due to the presence of residues 80-91 in the molecule /Table 1/.

TABLE 1 Biological and Biochemical Properties of Some Opioid Peptides

Peptide	Analgesic effect[+]	Bioassay index[++]	Helical potential[§]	Enzyme resistance[x]
Met-enkephalin /LPH^{61-65}/	1	0.04	-	0
δ-endorphin /LPH^{61-79}/	5	0.04	10	20
β-endorphin /LPH^{61-91}/	2500	0.84	60	60
/D-Met^{62}, Pro^{65}/-enkephalinamide	25000	1.05	-	80

[+] Reciprocal value of ED_{50} /μmol/animal/ as determined in the tail flick test after central administration /5,19/

[++] Ratio of the ID_{50} values determined in mouse vas deferens and guinea pig ileum /20,21/

[§] Percentage of α-helix as determined by CD spectroscopy in trifluoroethanol /5,22/

[x] Percentage of intact peptide in a 3-hour aminopeptidase M hydrolysate /for conditions of the digestion see Ref.5/

Two theoretical possibilities may be raised to explain the receptorial effect of this sequence portion: /a/ it contains additional binding site/s/, /b/ induces a favourable conformational change of the Met-enkephalin sequence of the polypeptide. In favour of the first alternative, it has been reported that LPH^{79-91} inhibits naloxone and dihydromorphine binding to opiate receptors with an IC_{50} of 3×10^{-6} M /24/. Furthermore, LPH^{80-91} gave an approximate K_e value of 3×10^{-5} M against normorphine in guinea pig ileum, whilst it had no opioid agonist activity at the same dose level. This weak antagonist

effect may be regarded as specific, because the inhibitory effect of norepinephrine was not antagonized by the peptide /A.Z. Rónai and L. Gráf, unpublished data/. As to the possible conformational effect of the C-terminal sequence portion in β-endorphin, there are two biochemical properties, e.g. the increased enzyme resistance and helical potential of the polypeptide /5,22, Table 1/, to support this view. Both phenomena are related to some conformational restraints in the molecule, most likely provided by hydrophobic bonding between the C- and N-terminal parts of β-endorphin /5/. Met^{65} in the N-terminal region and Lys^{88} and/or Lys^{89} of the C-terminus appear to have the highest capability to interact each other /for details see Ref. 5/.

We are aware of the possibility however, that features of a preferred solution conformation, like the non-polar intramolecular interaction suggested above, do not apply to the conformation assumed at the receptor site. The problem of receptor-bound conformation is an intriguing one, and the only approach to it is to correlate the biological and conformational /in solution/ effects of some amino acid substitutions. In this context it is remarkable that the selective oxidation of Met^{65} to methionine sulfone in the β-endorphin structure leads to the loss of biological activity in guinea pig ileum, mouse vas deferens and tail flick tests and also a considerable decrease of the helical potential of the molecule /A.Z.Rónai, J.I. Székely, M. Hollósi and L. Gráf, unpublished data/. Similarly, replacement of the same residue /Met^{65}/ by its D-isomer in β-endorphin results in a relatively inactive analog /25/. In contrast with this, the Met - D-Met substitution at position 65 of the enkephalin structure considerably improves the biological properties of the pentapeptide /8,21/. In fact, /D-Ala^{62}, D-Met^{65}/-enkephalinamide is a potent analgesic /8/. The above contradiction could be resolved by assuming a different biological role for Met^{65} in the enkephalin and β-endorphin structure. In enkephalin, Met^{65} and its substituents may be directly involved in receptor binding, as substantiated by the high bioassay index of the Pro^{65} enkephalin analogs /19,21, Table 1/. In the same time, Met^{65} of β-endorphin would rather participate in non-polar bonding with some residues, likely Lys^{88} or Lys^{89}, of the molecule to stabilize a newly formed binding site at the receptor surface. Thus our proposed model for the β-endorphin - receptor interaction /Fig. 2/ presumes a high degree of cooperativity in the binding process, i.e. mutual conformational adjustments of both the ligand and the receptor. This mode of ligand - macromolecule interaction has been formulated in the 'zipper' model /26/.

In mind of the delicate structural-conformational requirements for analgesic activity of β-endorphin, one is even more fascinated by the apparent similarities of some enkephalin analogs, like /D-Met^{62}, Pro^{65}/-enkephalinamide, to β-endorphin as regards their biological properties /Table 1/. Of the two amino acid substitutions in the enkephalin structure, the introduction of Pro at position 65 appears to produce an analogous receptorial effect /see the increment of the bioassay index in Ref. 21/ with the extension of the peptide chain to result β-endorphin. Pro^{65} of the enkephalin analogs thus, may mimic the binding site of the biologically active β-endorphin conformer /Fig. 2/. This however, would seem to require conformational adaptation of the complementary receptor site to the altered binding site in the ligand. In this context it is interesting to note that some *in vitro* bioassays clearly differentiate β-endorphin from /D-Met^{62}, Pro^{65}/-enkephalinamide /27/.

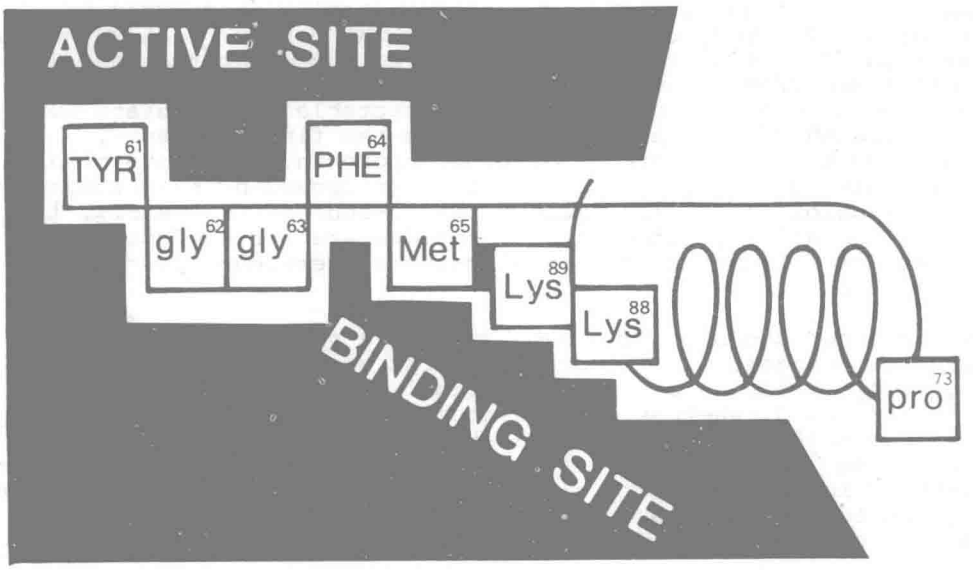

Fig. 2. Schematic diagram of the β-endorphin conformation assumed at the receptor surface. For further details see the text and Fig.9 of Ref. 5.

In the above part of my paper attempt has been made to explain the analgesic potency of natural and synthetic opioid peptides at the level of receptors. Indeed, we incline to give preference to the receptor binding affinity and specificity of opioid peptides over enzyme resistance, a common property of β-endorphin and synthetic peptide analgesics /5,28,29, Table 1/ that has been widely suggested to be responsible for their in vivo activities. Metabolic stability may really be an important factor contributing to the biological potency, however, not an exclusive one as demonstrated in one of our recent studies /29/. There is no doubt that the best peptide analgesics are those which carry structural features to account for more than just one favourable property. Namely, they should have improved receptor binding capacity and specificity, enhanced metabolic stability, favourable transport properties and also an ability to cross the blood-brain barrier, in the same time /29/.

PROTEINASES INVOLVED IN THE GENERATION OF PITUITARY ENDORPHINS

It is becoming increasingly apparent that all the secretory proteins are synthesized in the form of relatively large precursors, and that these precursors undergo intracellular proteolytic conversions into biologically active products /for reviews see Refs 30,31/. Evidence

for the high topographical and biochemical organization of this process within the cell has been obtained by the use of labeled amino acids in pulse-chase type experiments in vitro. Recently, such a biosynthetic pathway has been revealed for β-endorphin by applying the pulse-labeling technique and immunoprecipitation procedures in a mouse pituitary tumor cell line /32,33/. The curious feature of the above mechanism is that β-endorphin and adrenocorticotrophin /ACTH/ are processed from a common precursor with a molecular weight of about 31000. In addition, β-LPH as a detectable intermediate during the conversion of the large precursor to the final products, has been shown to be the direct precursor of β-endorphin. The above studies however, have not given useful information regarding the biosynthetic origin of opioid peptides, smaller than β-endorphin /see Fig. 1/. The question arises whether these peptides are formed from β-endorphin, and if so, whether these enzymatic conversions occur intra- or extracellularly.

β-LPH has been shown to be a substrate of a number of proteinases present in homogenates of porcine and rat adenohypophysis /34-37/. One of these proteinases, acting optimally at pH 4, splits the Leu^{77}-Phe^{78} bond, whereas another group of endopeptidases with a pH optimum of 8 attack the Lys^{46}-Met^{47}, Arg^{60}-Tyr^{61} and Lys^{79}-Asn^{80} bonds of the β-LPH structure. Thus, the occurrence of β-, γ- and δ-endorphins in the pituitary gland /see Fig. 1/ may be accounted for by the action of the above endopeptidases on β-LPH. γ-endorphin would be further processed to α-endorphin by a carboxypeptidase /34/.

Of the subcellular fractions obtained by differential centrifugation of a porcine adenohypophysis homogenate, fraction P_2 /38/ shows the highest β-LPH hydrolysing activity as tested at either pH 4 or pH 8 /37, Table 2/. The different resistance of the P_2 fraction enzymes to osmotic lysis clearly indicates that they are associated with different compartments of the cell. Fraction P_2 is known to be composed of mitochondria, secretory granules and lysosomes /38, 39/.

TABLE 2 Subcellular Localisation of Two β-LPH Converting Enzymes in Porcine Pituitary

Fraction	Endopeptidase splitting Arg^{60}-Tyr^{61}	Endopeptidase splitting Leu^{77}-Phe^{78}
P_1 /1 x 10^4 g min/	+	+
P_2 /3 x 10^5 g min/	+++	+++
P_3 /3 x 10^6 g min/	+	+
S	-	+
P_2 — Lysis — P_2P /3 x 10^6 g min/	+++	+
P_2 — Lysis — P_2S	-	+++