A MOLECULAR BASIS FOR THE INTERACTION OF CORTICOTROPIN WITH OPIATE RECEPTORS

Christopher R. SNELL and Penelope H. SNELL
MRC Neuroendocrinology Unit, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne NE4 6BE, England

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1. Introduction

Earlier investigations have shown corticotropin (ACTH) to produce pharmacological effects previously thought to be specific for opioid peptides and alkaloids. Corticotropin peptides and opioid peptides both produce characteristic grooming behaviour in rats on intraventricular injection [1], and have also been shown to inhibit the electrically evoked contraction of the mouse vas deferens in vitro [2] in a naloxone reversible manner. More recently, corticotropin related peptides were found to produce similar analgesic effects to β-endorphin on injection directly into the periaqueductal gray of the rat [3]. These observations along with the report [4] that corticotropin 1–24 can displace [^{3}H]naloxone from opiate receptors in rat brain membranes strongly suggests that corticotropin peptides can interact with the opiate receptor. This paper describes a structure activity study of the opiate receptor interaction of corticotropin and related peptides using [^{3}H]dihydromorphine and [^{3}H]naloxone as labelled ligands. The data is correlated with the predicted receptor conformation of the enkephalins and corticotropin and explains the molecular basis for the interaction of corticotropin with the opiate receptor, suggesting that the receptor environment induces the N-terminal region of corticotropin into an α-helical conformation.

2. Materials and methods

2.1. *Peptides*

Corticotropin was isolated from porcine pituitary glands as in [5]. The peptides Gln^{5}ACTH (1–10) amide, Gln^{5}ACTH (2–10) amide and Gln^{5}ACTH (3–10) amide was synthesised by solid-phase methodology [6]. The peptide resins, BOC.Ser(Bzl).Tyr(Bzl).Ser(Bzl).Met.Glu(Bzl).His.Phe.Arg(Tos).Trp.Gly-resin and the corresponding peptidoresins for the 2–10 and 3–10 analogs, were treated with anhydrous methanol saturated with ammonia to remove the peptide from the resin as the protected Gln^5 amides. The protected peptides were purified by gel filtration through Sephadex LH-20 eluted with dimethylformamide. The purified peptides were deprotected by treatment with trifluoroacetic acid followed by sodium in liquid ammonia reduction in the presence of excess free tryptophan. The peptides were finally purified by gel filtration through Sephadex G-25 eluted with 50% acetic acid. The resulting peptides had the required amino acid compositions after hydrolysis in 6 M HCl containing 1% phenol.

2.2. *Tissue preparation*

Sprague-Dawley rats (250 g) were decapitated and the brains without cerebellum were rapidly removed. Tissue was homogenised in 20 vol. ice cold 0.32 M sucrose in a Potter glass homogeniser fitted with a Teflon pestle. The whole homogenate was centrifuged at 1000 × *g* for 15 min at 4°C; the pellet was discarded and the supernatant was centrifuged at 50 000 × *g* for 30 min at 4°C. The resultant pellet was hypotonically lysed and washed 3 times in 0.01 M Tris–HCl (pH 7.4). The final pellet was suspended in this buffer, divided into small aliquots and stored at −20°C.

2.3. *Receptor binding assay*

To aliquots of membrane suspension (1 mg protein), in 0.05 M Tris–HCl, 0.1 M NaCl, 0.1% BSA and 0.01% bacitracin (pH 7.4) buffer, was added [^{3}H]-naloxone (50 Ci mmol^{-1}, final conc./tube 1 nM) or [^{3}H]dihydromorphine (73 Ci mmol^{-1}, final conc./

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tube 0.5 nM) in the presence of known concentrations of test peptide; the final volume of the incubation mixture was 1 ml. After incubation at 30°C for 15 min the suspension was centrifuged (16 000 × *g*; 3 min), the supernatant removed by aspiration and the pellet superficially washed with ice cold 0.05 M Tris–HCl, 0.1 M NaCl (pH 7.4) buffer. The tips of the tubes containing the pellet were removed and the pellet resuspended in 0.5 ml water. After addition of 6 ml Packard 299 Scintillation fluid the radioactivity was assayed by liquid scintillation counting at 30% efficiency.

2.4. *Conformational predictions*

The methods used were essentially those in [7,8] for the prediction of secondary structure in proteins.

3. Results and discussion

The structural requirements for the stimulation of the opiate receptor by opioid peptides has been extensively studied using synthetic analogs [9] but these studies alone have given little insight into the receptor conformation of the enkephalins. Theoretical approaches [10,11], however, have proved a useful starting point for evaluating structure activity data in terms of receptor conformation. The empirical methods of Chou and Fasman [7,8] for predicting protein conformation have been used to predict the conformation of small peptides in the proteinaceous environment of their receptors [12], and for enkephalin, a β-bend at the 1–4 position has been proposed [11,12]. Support for this hypothesis has come from the X-ray crystallography of leucine–enkephalin showing the presence of such a β-bend [13]. In this conformation it is possible to explain the ability of the opioid peptides and alkaloids to interact with the same receptor. There is a remarkable similarity in the orientation of functional groups in the peptides and morphine alkaloids, particularly the very potent oripavines [11]. The similarities in methionine–enkephalin reside with the tyrosine sidechain and α-amino group in position 1, the phenylalanine sidechain in position 4 and the methionine sidechain in position 5.

The ability of corticotropin to bind to the 'opiate' receptor should involve some or all of these determinants. The possibility that the tyrosine sidechain at position 2 in corticotropin could correspond to the tyrosine in enkephalin at position 1 which is essential for opiate receptor interaction is examined using synthetic peptides corresponding to Gln^5ACTH(1–10)NH_2, Gln^5ACTH(2–10)NH_2 and Gln^5ACTH(3–10)NH_2. The Gln^5 substitution in corticotropin has little effect on biological activity [14] and was used in this work to facilitate the solid phase synthesis:

H.Ser.Tyr.Ser.Met.Gln.His.Phe.Arg.Trp.Gly.NH_2 Gln^5ACTH(1–10)NH_2
1 2 3 4 5 6 7 8 9 10

H.Tyr.Gly.Gly.Phe.Met.OH Methionine–enkephalin
1 2 3 4 5

Gln^5ACTH(2–10) has Ser^1 missing and consequently the α-amino group of the Tyr^2 exposed, and Gln^5ACTH(3–10)NH_2 has the Tyr^2 residue under investigation removed. Table 1 shows the ability of these peptides, as well as corticotropin (1–39) and the opiate alkaloids and peptides, to displace [^{3}H]-naloxone and [^{3}H]dihydromorphine from rat brain synaptosomal membranes. Corticotropin (1–39) shows a significant preference for the dihydromorphine-labelled receptor with an affinity as high as those reported for some of the less active enkephalin analogs [9]. Removal of residues 11–39 as in Gln^5ACTH(1–10)NH_2 leads to a reduction of affinity but clearly shows the 11–39 residues of corticotropin not to be essential for interaction with the opiate receptor; these residues do however appear to be responsible for the discrimination of corticotropin between the dihydromorphine and naloxone labelled receptors. The receptor affinity of Gln^5ACTH(2–10)NH_2 is 3–4-fold higher than Gln^5ACTH(1–10)NH_2 for both ligands. This would be expected if Tyr^2 in corticotropin were equivalent to Tyr^1 in enkephalin,

Table 1
Concentrations for 50% inhibition of [^{3}H]dihydromorphine and [^{3}H]naloxone binding to brain membranes by corticotropin, corticotropin related peptides, opioid peptides and alkaloids

	[^{3}H]Dihydromorphine (nM)	[^{3}H]Naloxone (nM)
ACTH	0.1	0.75
Gln^5ACTH(1–10)NH_2	11	16
Gln^5ACTH(2–10)NH_2	3	7
Gln^5ACTH(3–10)NH_2	20	35
Met-Enkephalin	0.008	0.009
β-Endorphin	0.0022	0.0026
Dihydromorphine	0.0036	0.002
Naloxone	0.0024	0.0018

since removal of Ser^1 from corticotropin exposes the α-amino group of Tyr^2 found in enkephalin and known to be important for opiate receptor binding [9]. The equivalence of the 2 tyrosines is also demonstrated by the dramatic drop in receptor affinity when Tyr^2 is subsequently removed, as in $Gln^5(3-10)NH_2$. The changes in opiate receptor affinity of Gln^5ACTH-$(1-10)NH_2$ when Ser^1 and Tyr^2 are removed parallel those observed when, for instance, the Glycyl and Tyr^1 residues are removed from *N*-glycyl-methionine–enkephalin [15]. Therefore, it would appear that Tyr^2 in corticotropin serves the same function as Tyr^1 in enkephalin at the opiate receptor.

Extensive structure activity work on the hormonal activity of corticotropin [16] has shown that the core sequence which is vital for corticotropin receptor stimulation is contained in the N-terminal 10 residues; consequently, these residues should be those principally involved in receptor interaction. This area of corticotropin is predicted to be helical in the proteinaceous environment of the receptor [12] using the conformation prediction methods in [7,8].

If Tyr^2 in corticotropin in an α-helical conformation is aligned with Tyr^1 in a β-bend conformation there is a remarkable similarity in orientation of identical sidechains in the 2 molecules; these conformations are shown diagramatically in fig.1. Tyr^2, Met^4 and Phe^7 sidechains in corticotropin fall in exactly the same spacial orientation as Tyr^1, Phe^4 and Met^5 in enkephalin, these being the residues essential for the biological activity of enkephalin at the opiate receptor.

We conclude from these results that it is the N-terminal region of corticotropin that interacts with the opiate receptor and it is the structural similarity of this region of corticotropin in an α-helical conformation with the receptor conformation of enkephalin that accounts for this overlap in activity. The physiological receptor for corticotropin in the central nervous system has yet to be demonstrated, however, it is probably closely involved with the opiate receptor in view of the common biosynthetic origin of the endorphins and corticotropin [17–20] and the similar distribution of these peptides in the central nervous system [21], probably within the same neuron.

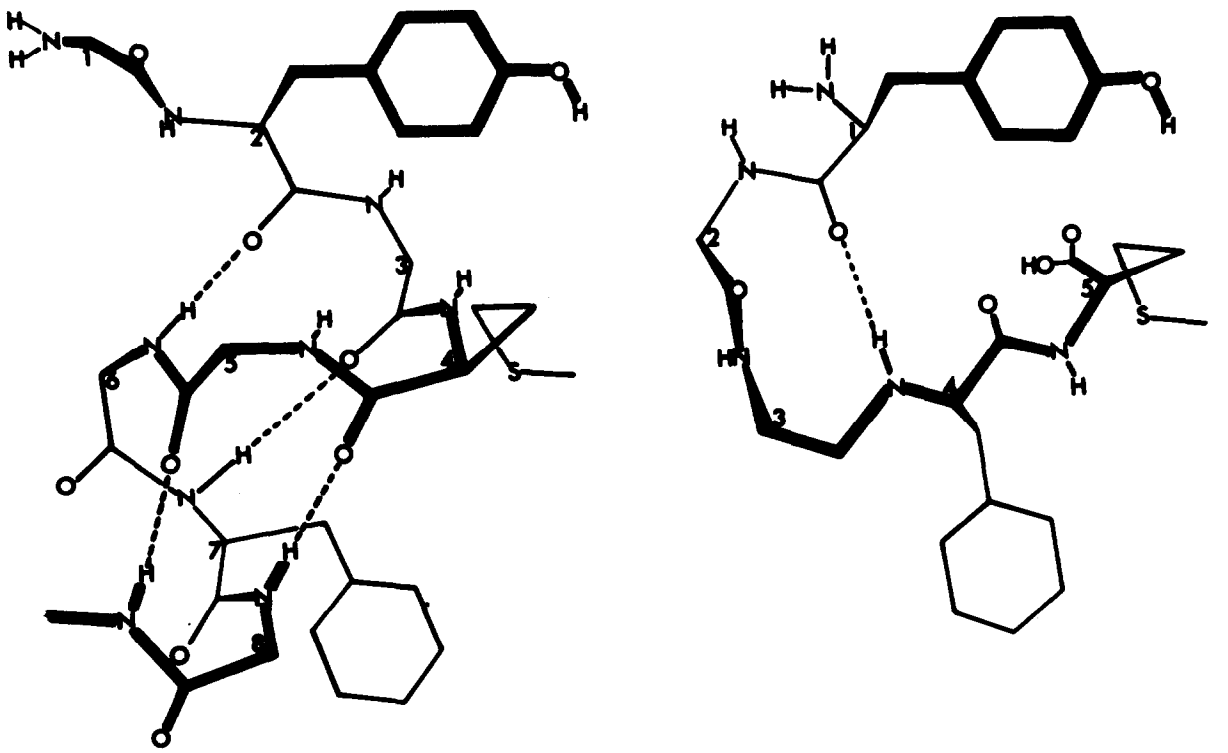

Fig.1. Comparison of the predicted α-helical conformation of the N-terminal region of corticotropin (left) and the predicted β-bend conformation of methionine enkephalin (right). The numbers refer to the residue numbers of the 2 molecules, only the relevant sidechains are shown for clarity.

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