Relationship between primary structure and activity in exorphins and endogenous opioid peptides

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We have found a correlation between the certain characteristics of primary structure and biologic activity in exorphins and endogenous opioid peptide family. The characteristics of primary structure are the content of certain segment pairs as well as the density of their arrangement in a peptide. These segment pairs represent basic elements of the regulatory peptide primary structure pattern, which was found recently [Dokl. Akad. Nauk USSR 289 (1986) 721–724; Int. J. Peptide Prot. Res. 38 (1991) 505–510].

1. INTRODUCTION

In recent papers the pattern found for amino acid alternations in regulatory peptides was reported [1,2]. Regulatory peptides were represented as a sequence of hydrophobic and hydrophilic segments. The segments were respectively classified into 2 and 3 different types according to the peculiarities of mutual localization of hydrophobic and hydrophilic amino acid residues within the peptide primary structure. When compared with proteins, nonregulatory peptides and a random sequence of segments, the regulatory peptides were characterized by an increased frequency of 4 particular pairs of segments among 12 theoretically possible pairs. These 4 pairs represent the fragments of a periodical segment sequence with a period of 4. The observed pattern indicates that there exists a general principle for the primary structure organization of regulatory peptides and possibly a common type of their flexible peptide chain folding at the ligand–receptor complex formation. However, causal relationship of the pattern and the ability of regulatory peptides for specific binding to receptors is still unclear.

To analyze how certain segment combinations in regulatory peptides are related to their biologic activity we investigated primary structure–activity relationship using a statistical approach. We have attempted to find a relationship between the content of the characteristic pairs of segments and the biological activity of exorphins and endogenous opioid peptides. Thus, the largest group among biologically active peptide families was chosen for analysis.

For a more detailed consideration of the regulatory peptide primary structure pattern we should also describe here the features of mutual localization of amino acid residues and the classification of the segments in regulatory peptides. It was found [1,2] that hydrophobic residues Pro, Tyr, Ile, Val, Trp and Cys (group P′) more often precede an adjacent Gly or Asn (group G′ of hydrophilic residues) than follow them. The G′ residues more frequently precede, than follow another group of hydrophobic residues (Phe, Leu, Ala and Met - group F′). The F′ group more frequently precedes Arg, Ser, Thr and Lys (group R′ of hydrophilic residues) rather than follows them, and, finally, R′ group more frequently precedes than follows the P′ group. The segments of regulatory peptides were classified into 2 hydrophobic (<P′>, <F′>) and 3 hydrophilic (<G′>, <R′> and <B′>) types according to their amino acid content [1,2]. Segment <P′> contains P′, and other hydrophobic residues; segment <F′> consists of F′ residues; segment <G′> contains G′ and other hydrophilic residues; segment <R′> contains R′ and other hydrophilic residues, but not G′, segment <B′> consists of Glu, Gln, His, and Asp. We have observed that regulatory peptides are characterized by an increased frequency of 4 particular pairs of segments <P′>-<G′>, <G′>-<F′>, <F′>-<R′> and <R′>-<P′>) which are the fragments of periodical segment sequence -<P′>-<G′>-<F′>-<R′>-<P′>-. In the present study we have used this amino acid and segment classification to reveal (a) the features of the primary structure of the peptides

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interacting with opioid receptors and (b) an association between the features and biological activity of the peptides.

2. METHODOLOGY, METHODS AND DATA

The peptides chosen for this study are naturally occurring opioids, exorphins and endogenous opioid peptides consisting of L-amino acid residues and exerting their action through opioid receptors (Table 1).

Recent studies [1,2] have demonstrated that with regard to their chain-length distribution, regulatory peptides may be divided into two groups: the group of short regulatory peptides with a length of up to 20 residues and the group of long regulatory peptides containing over 20 residues. The pattern of segment alternations was revealed for the short regulatory peptides. Long regulatory peptides do not fit to the pattern so well: the quantitative differences between this group and a random segment sequence were weaker compared to the group of short peptides. Therefore, in further considerations only peptides containing less than 20 residues were selected.

In accordance with our previous approach [1,2] we here classify residues Pro, Tyr, Val, Ile, Trp, Cys as P'; Phe, Leu, Ala, Met as F'; Arg, Ser, Thr, Lys as R'; Gly, Asn as G'; Asp, Glu, His, Gln as B'. The segments cntaining P' and F' are referred to as <P'>; containing F' as <F'>; containing G', R' and B' as <G'>; those containing R' and B' as <R'>, and those consisting of B' as <B'>.

To characterize the ability of the peptides to interact with opioid receptors we have used IC_{50} and K_i (or K_d) values obtained in pharmacological assays on isolated electrically stimulated tissues (guinea pig ileum (GPI), mouse or rat vas deferens (M/RVD)), and in biochemical binding assays, respectively (Table 1). To reveal the general properties of the peptides in the sample we have only taken the maximal value of biological activity obtained in either one of three isolated tissue preparations or in the binding assays described. The maximal value of activity reflects the principal ability of peptides to interact with receptors independently of whether there are several types of opioid receptors or not. The mean value of the parameters was calculated using data from the papers indicated (Table 1).

Correlations were generated by regression analysis fitting the best straight line via the least-squares method and a t-test was used in order to test the significance of the observed association. Since we have no evidence that the scores used were from a bivariate normal population we also applied a nonparametric technique for measuring the degree of correlation between the different variables (Spearman rank correlation coefficient, rs) and its significance.

3. RESULTS AND DISCUSSION

Table II shows the segment pairs of opioid peptides which occur at a higher rate than in a random sequence

Table I

Exorphins and endogenous opioid peptides. Amino acid and segment sequences and biological activity

Peptides	Amino acid sequence	Segment sequence	Characteristic segment pairs, No.	Biological activity*		References
				GPI, M/RVD, $-\log[IC_{50}]$ (M)	Binding assay, $-\log[K_{id}]$ (M)	
Cytochrophin-4	YPFT	<P'R'>	0	3.9	-	4,5
Hemorphin-4	YPWT	<P'R'>	0	4.4	-	5
Morphiceptin	YPFP-NH₂	<P'>	0	6.2	7.5	3,7,18
h-β-Casomorphin-5	YPFVE	<P'B'>	0	4.6	4.7	4,14
b-β-Casomorphin-5	YPFPG	<P'G'>	1	5.9	6.4	4,5,7,14
Cytochrophin-5	YPFTI	<P'R'P'>	1	3.5	-	4,5
Hemorphin-5	YPWTE	<P'R'>	0	4.3	-	5
Leu-enkephalin	YGGFL	<P'G'F '>	2	8.0	8.5	6,8,10,15,23,24,27
Met-enkephalin	YGGFM	<P'G'F'>	2	7.8	8.7	6,9,15,19,24,25,27
α-Casein (90–95)	RYLGY	<R'P'G'P'>	2	4.2	6.1	16
α-Casein (91–96)	YLGYL	<P'G'P'>	1	-	5.0	16
Leu-enkephalinR⁶	YGGFLR	<P'G'F'R'>	3	7.5	-	10,17,19,22,23
h-β-Casomorphin-7	YPFVEPI	<P'B'P'>	0	4.5	5.2	14
b-β-Casomorphin-7	YPFPGPI	<P'G'P'>	1	5.3	4.6	7,14
α-Casein (90–96)	RYLGYLE	<R'P'G'P'B'>	2	4.5	6.8	16
Met-enkephalinR⁶F⁷	YGGFMRF	<P'G'F"R'F'>	3	8.3	7.6	17,19,27
h-β-Casomorphin-8	YPFVEPIP	<P'B'P'>	0	4.8	5.2	14
b-β-Casomorphin-8	YPFPGPIP	<P'G'P'>	1	5.5	4.2	14
Met-enkephalinR⁶G⁷L⁸	YGGFMRGL	<P'G'F'G'F'>	3	8.5	8.3	17,19
Dynorphin A (1–8)	YGGFLRRI	<P'G'F'R'P'>	4	8.3	8.9	10
Metorphamide	YGGFMRRV-NH₂	<P'G'F'R'P'>	4	8.6	9.9	26
β-Neoendorphin	YGGFLRKYP	<P'G'F'R'P'>	4	7.9	8.9	12,21,22
α-Neoendorphin	YGGFLRKYPK	<P'G'F'R'P'R'>	4	7.9	9.7	10,12,21
Dynorphin B	YGGFLRRDFKVVT	<P'G'F'R'F'R'P'R'>	5	8.8	9.9	12,13,22
Dynorphin A (1–13)	YGGFLRRIRPKLK	<P'G'F'R'P'R'P'R'F'R'>	6	9.1	10.3	8,10–12,20
α-Endorphin	YGGFMTSEKSDTPLVT	<P'G'F'R'P'R'>	4	7.6	7.0	9,10,21,25
γ-Endorphin	YGGFMTSEKSDTPLVTL	<P'G'F'R'P'R'F'>	4	7.6	7.2	21,25
Dynorphin A (1–17)	YGGFLRRIRPKLKWDNQ	<P'G'F'R'P'R'P'R'F'R'P'G'>	8	9.5	9.9	10,22

*The maximal values of the parameters were selected or mean maximum values of those were calculated using data from the papers indicated.

Table II

The segment pairs of which the content in opioid peptides, as well as in regulatory peptides, exceeds that in random segment sequences (" data taken from [1,2]). The ratio of segment pair frequency in the peptides to segment pair sequence in a random segment sequence is given. The relative content of segments of various types in a random segment sequence and in opioid peptides was assumed to be the same. Analysis was performed and data are presented as described previously [2] (Table III): *P < 0.01, **P < 0.001, ***P < 0.0002.

Segment pair type	Opioid peptides	Regulatory peptides"
<P'>-<G'>	1.34**	1.20***
<G'>-<F'>	1.58**	1.38***
<F'>-<R'>	1.87**	1.59***
<R'>-<P'>	1.43*	1.26***

of segments. When compared to a random segment sequence, opioid peptides (like short regulatory peptides [1,2]) have a significantly higher occurrence rate of the following segment pairs: <P'>-<G'>, <G'>-<F'>, <F'>-<R'> and <R'>-<P'>. In conformity with what was found for the short regulatory peptides these pairs

Table III

Relationship between biologic activity (−log [IC$_{50}$ or K_i (K_d)]) and properties of primary structure of the peptides. Coefficient of correlation (r) and Spearman rank correlation coefficient (rs; values corrected for ties are shown in brakets).

Numbers (n)/peptide	Activity in tissue preparations		Activity in binding assay	
	r	rs	r	rs
Amino acids, n	0.59	0.62	0.40	0.44
Segment pairs, n	0.70	0.67	0.63	0.62
Characteristic segment pairs, n	0.86	0.80 (0.84)	0.80	0.79 (0.82)
Ratio of n of segment pairs/ n of amino acids	0.59	0.57	0.57	0.58
Ratio of n of characteristic segment pairs/n of amino acids	0.85	0.76 (0.79)	0.82	0.85 (0.85)
Ratio of n of characteristic segment pairs/n of segment pairs	0.79	0.65	0.70	0.64

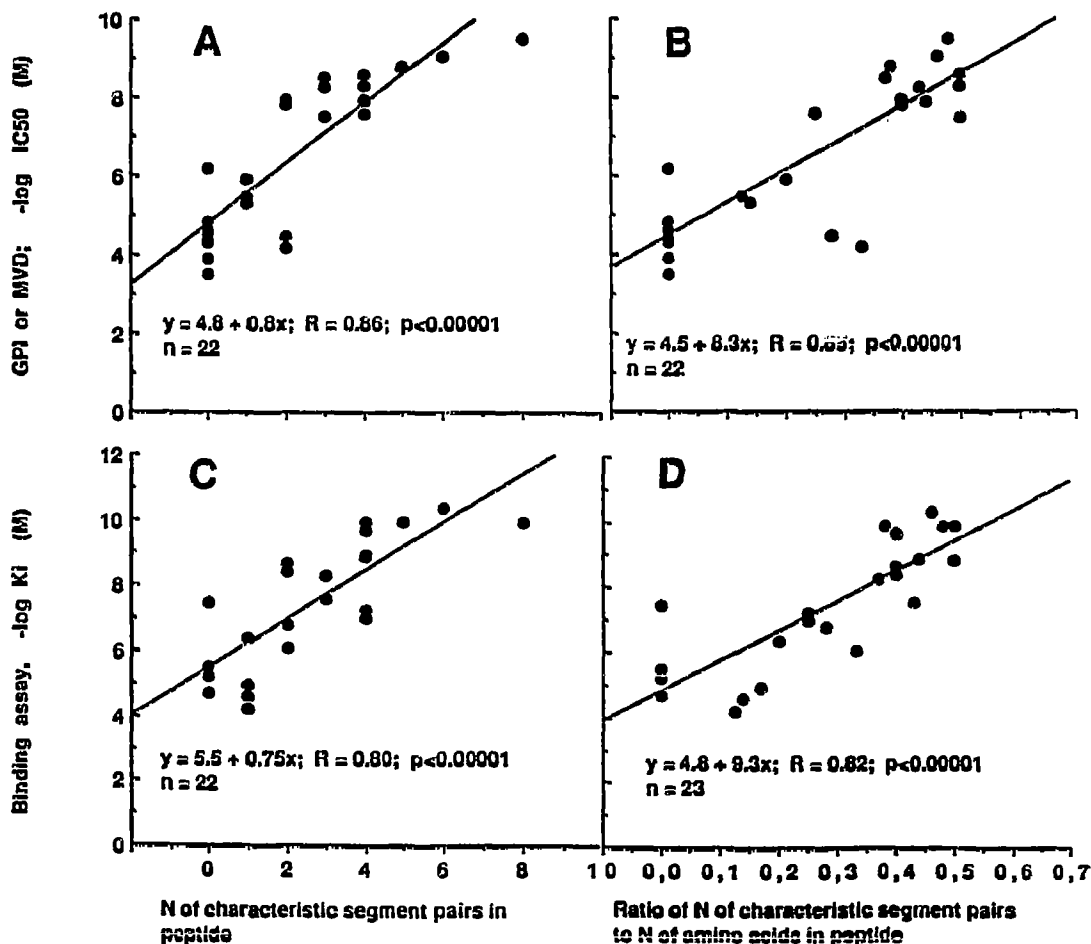of segments are the fragments of a hypothetic periodical sequence -<P'>-<G'>-<F'>-<R'>-<P'>.

Fig. 1. Relationship between the biological activity and the number of characteristic segment pairs in peptide (A,C) or the ratio of the number of characteristic segment pairs to the number of amino acid in peptide (B,D). The IC$_{50}$ values (A,B) were determined in pharmacological assays on isolated tissue and K_i (or K_d) values were determined in biochemical binding assays (data were taken from the papers indicated, see Table I).

The analysis of the relationship between the biological activity of the peptides and parameters describing the properties of their primary structure showed that the best association occurs between the biological activity and the number of characteristic segment pairs in the peptide ($r = 0.80$–0.86, $P < 0.00001$; $rs = 0.79$–0.80, $P < 0.001$) (Fig. 1; Table III). This holds true for the relationship of the biological activity and the ratio of the number of characteristic segment pairs to the number of amino acid residues in peptide ($r = 0.82$–0.84, $P < 0.00001$; $rs = 0.76$–0.85, $P < 0.001$) (Table III, Fig. 1).

Thus, the higher the number of characteristic segment pairs in the opioid peptide, the higher its biological activity (receptor affinity). The same is found for the ratio of the number of characteristic segment pairs and the number of amino acids in the peptide (i.e. the density of arrangement of characteristic segment pairs along a peptide sequence) and the biological activity. It has been assumed that the peptide containing the observed periodical sequence of segments may form a 'regular' three-dimensional structure [2]. The structure of each regulatory peptide at the ligand–receptor complex may be a fragment of this 'regular' structure. Regarding the present results, it seems likely that the higher the number and density of characteristic segment pairs in a peptide, the better it can fit to this three-dimensional structure, resulting in higher biological activity.

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