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SYNTHESIS OF PEPTIDE FRAGMENTS OF NEUROPEPTIDE Y: POTENT INHIBITORS OF CALMODULIN-STIMULATED PHOSPHODIESTERASE

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Peptide fragments of neuropeptide Y (NPY) were synthesized by the solid phase method. Carboxy-half fragments were more potent inhibitors of calmodulin-stimulated phosphodiesterase than NPY, but amino-half fragments were not inhibitory. Circular dichroism was studied to determine the secondary structures of the peptide fragments. The basic amphiphilic helical nature of the carboxy-half region of NPY was responsible for its CaM inhibitory effect. Further structure-activity relationships were studied by amino acid(s) replacement.

KEYWORDS —— calmodulin; inhibitor; neuropeptide Y; peptide fragment; solid phase synthesis; structure-activity relationship; amphiphilicity; α-helix

Calmodulin (CaM) is a ubiquitous calcium binding protein that regulates many enzymatic and cytoskeletal systems calcium-dependently. Compounds that inhibit the action of the Ca-CaM complex can regulate various cellular functions without changing the intracellular calcium concentration (cf. Ca-channel blocker).

Some endogenous peptides (e.g. endorphin, VIP) inhibit CaM. Their structure-activity relationships were studied and some of their fragments were also CaM-inhibitory.[1] However, none of the fragments were more potent inhibitors than the original endogenous peptides.

Neuropeptide Y (NPY) (Fig. 1) is an important neurotransmitter that regulates the cardiovascular system. It is especially interesting that NPY is CaM-inhibitory (IC_{50} = 0.5μM).[2]

YPSKPDNPGEDAPAEDLARYYSALRHYINLITRQRY-NH_2

Fig. 1. Neuropeptide Y (NPY)

To obtain a novel cardio-regulator, we synthesized peptide fragments of NPY, and some fragments were more potent than NPY. The CaM-inhibition of the peptide fragments and their structure-activity relationships are described here.

Peptides were synthesized by the solid phase method. Side chain functional

groups and α-amino groups of amino acids were protected with the benzyl type and t-BOC protecting groups respectively. Amino acids were sequentially coupled on 4-hydroxymethylphenylacetamidomethyl (PAM) resin or p-methylbenzhydrylamine (BHA) resin. The peptide-resins were finally deprotected by the high HF procedure. Crude peptides were purified by preparative reversed phase HPLC and then lyophilized. Satisfactory mass spectra and amino acid composition analysis data were obtained for all of the new compounds in this paper. The potency of the inhibition of CaM-stimulated phosphodiesterase by the peptides was measured by the luciferin-luciferase method.[3] The potencies (IC_{50}) are shown in Tables I and II.

Basicity and hydrophobicity are important factors in CaM inhibitors, because CaM is an acidic hydrophobic protein. The carboxy-half (C-half) of NPY comprises basic amino acids and hydrophobic amino acids, but the amino-half (N-half)

Table I. CaM Inhibitory Potencies of NPY and Peptide Fragments

No.	Sequence	IC_{50} (μM)	Helical content (%)
NPY	YPSKPDNPGEDAPAEDLARYYSALRHYINLITRQRY-NH2	0.5	37
1	YPSKPDNPGEDAPAEDLARYYSAL-OH	>50	11
2	YPSKPDNPGEDAPA-OH	>50	3
3	APAEDLARYYSALRHYINLITRQRY-NH2 (22–33)	0.06	47
4	SALRHYINLITRQRY-NH2	0.05	40
5	SALRHYINLITR-NH2	0.2	33
6	SALRHYINLIT-NH2	2	30
7	ALRHYINLITR-NH2	0.7	30

comprises acidic amino acids and hydrophilic amino acids. The N-half fragments (1, 2) were not potent. Unexpectedly, the C-half fragments (3, 4) were more inhibitory than NPY (Table I). An amphiphilic helical structure is also an important factor in CaM-inhibitory peptides.[4] The residues from 14 to 31 of avian pancreatic polypeptide (APP) that has sequence homology with NPY have an α-helical structure.[5] The structure of APP is stabilized by a number of intramolecular hydrogen bonds, ionic interactions, and hydrophobic interactions. The circular dichroic spectra of NPY fragments that had fewer intramolecular interactions than intact NPY were measured in 70% trifluoroethanol to determine their α-helical contents. NPY had 37% α-helical content. Fragment 1, containing an estimated α-helical region of NPY, had little α-helicity. Potent fragments 3 and 4 had higher α-helical contents than NPY. A helical axis projection of 3 indicates that there is a polar face and an apolar face (Fig. 2). The apolar face interacts with the N-terminal polyproline-like helix (residues from 1 to 8) of NPY and is shielded from water.[6] The removal of the N-terminal region from NPY reduced the intramolecular ionic interactions between the acidic residues in the N-terminal region and the basic residues in the C-half region, and exposed the apolar face. So 3 interacted more with CaM than NPY.

To prepare short chain inhibitors, amino acid(s) was (were) removed from 4 (Table I, 5-7). The contribution of the three C-terminal residues of 4, containing a basic Arg residue, to the interaction with CaM may be little, because they did not match the amphiphilicity pattern of the residues from 22 to 33. So the potency was little affected by their removal, and 5 was the smallest peptide that had a higher potency than NPY. The removal of either the N-terminal Ser residue or the C-terminal Arg residue from 5 weakened the potency, even though the secondary structure was not affected by these removals (6, 7). Decrease of amphiphilicity due to the removal of hydrophilic Ser, or decrease of basicity due to the removal of Arg from the polar face was responsible for their low potencies. These results suggest that not only hydrophobic or basic residues but also a hydrophilic residue at an appropriate position, like Ser^{22}, have an important effect on the potency.

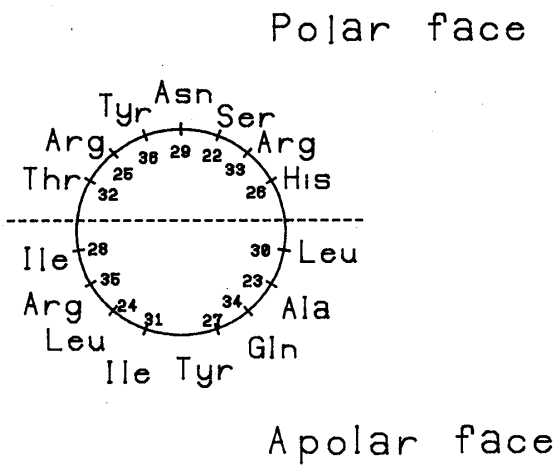

Fig. 2. Helical Axis Projection of NPY (22-36) (3)

Table II. CaM Inhibitory Potencies of Peptide Fragments of NPY

No.	Sequence (^{22}...^{33})	IC_{50} (μM)
8	GALRHYINLITR-NH2	0.5
9	SGLRHYINLITR-NH2	0.2
10	SAGRHYINLITR-NH2	10
11	SALGHYINLITR-NH2	5
12	SALRGYINLITR-NH2	0.3
13	SALRHGINLITR-NH2	5
14	SALRHYGNLITR-NH2	5
15	SALRHYIGLITR-NH2	1
16	SALRHYINGITR-NH2	1
17	SALRHYINLGTR-NH2	0.8
18	SALRHYINLIGR-NH2	0.5
19	SALRHYINLITG-NH2	3
20	SALKHYINLITK-NH2	0.5
21	SALRHFINLITR-NH2	0.5
22	SALRHYIQLITR-NH2	0.3

Fragments 8-22 were prepared to study further structure-activity relationships (Table II). In the N-terminal region of NPY (22-33), Ser^{22} or Ala^{23} was replaceable without decreasing the potency (8, 9). However, the replacement of Leu^{24} with Gly residue greatly reduced the potency (10). Not only was hydrophobic Leu^{24} important, but the existence of two residues on its N-terminal was also important for the interaction with CaM (7). Ala^{23} was the non-hydrophobic residue on the apolar face. It did not directly contribute to the interaction with CaM. Thus this position can be occupied by either a hydrophilic residue or a hydrophobic residue, and is a candidate for further modifications.

When a residue was replaced with an amino acid which had a hydrophobicity value[7] close to the former, there was little loss of the potency: Ser, Ala, His, or Thr with Gly (8, 9, 12, 18); Arg with Lys (20); Tyr with Phe (21). The hydroxyl group of Tyr was not important for the potency. But the side chain amide

group at the 29th position was important. The replacement of Asn^{29} with the Gly residue reduced the potency in spite of the close hydrophobicity values of Asn and Gly (15). The replacement of Asn^{29} with the Gln residue had little effect on the potency (22). When a residue was replaced with an amino acid with a different hydrophobicity, the potency was reduced: Leu, Tyr, or Ile with Gly (10, 13, 14, 16, 17); Arg with Gly (11, 19). These results suggest that the original hydrophobicity profile should be maintained in further modifications of NPY.

In conclusion, short chain, potent CaM-inhibiting derivatives of NPY were obtained, and this is the first example of a derivative of endogenous peptide exhibiting a higher potency than the lead compound. We have presented a guide for further modification of NPY, the importance of the amphiphilic pattern, and the contributions of residues to the potency. Studies of the biological activities of these short chain derivatives of NPY are in progress.

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