ALANINE-SCANNING OF THE NEUROPEPTIDE VIP: MOLECULAR MODELING AND EXPERIMENTAL APPROACHES ALLOW TO DISCRIMINATE THE RESIDUES IMPORTANT FOR STRUCTURE AND/OR ACTIVITY.

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VIP is a 28-residue neuropeptide which binds to the extracellular N-terminal domain of the VPAC1 receptor, a G-coupled protein (RCPG) containing seven putative transmembrane segments, belonging to the RCPG class II (1). In order to define which residues are implicated in the function and/or in the structure, all VIP residues were successively mutated to alanine: each mutation ending to one different analog. All analogs were synthesized and their abilities to bind the VPAC1 receptor and to stimulate the adenylyl cyclase were measured. The 3D structures of the VIP and of all analogs were calculated by molecular modeling using OSIRIS (2,3). OSIRIS is an «ab initio» method which uses the primary sequence as unique input. The calculation has two successive steps: in the first step 179 phi/psi angles representative of the Ramachandran plot are imposed to each residue. The molecule is then built from the amino acid combination of the lowest energies. The second step is angular molecular dynamics of this structure.

In the ala-scan of the VIP, only the first step of OSIRIS is used with one modification to test structure variability. The amino acid combination of energy minima is calculated by an iterative process. The first step of OSIRIS is run once and only μ residues are structurally frozen: the μ residues of lowest energies. Then step 1 is iterated up to the freezing of the whole molecule; μ varied between 5 and 28. For each μ, one structure was obtained and thus one analog corresponded to several structures. The statistical dispersion of those structures was calculated using the SICLA program (clustering procedure). Thus for each sequence, we determined a median structure and defined whether the X-A mutation induced structural variability with respect to the WT.

The WT VIP structure is essentially a α helical from Val5 to Asn 24. The structural variability observed with the different μ is observed for the C-terminal domain (25 to 28). Structural differences are observed for some Ala mutants with respect to the WT, either for the median structures or for the structural dispersion or for both. Those mutants are : D3, F6, T7, D8, Y10, R12, L13, K15, K20. Other mutations do not induce any structural modification. In the biological tests, mutation of : H1, D3, V5, F6, T7, D8, Y10, R12, L13, R14, K15, K20, Y22, L23, I26 decreases the binding to the VPAC1 receptor and the cyclase activity. Other mutations, i.e. S2, A4, N9, T11, Q16, M17, A18, V19, K21, N24, S25, L27, N28 do not affect the biological activity.

By combining both approaches, we can observe that : the mutations of S2, A4, N9, T11, Q16, M17, A18, V19, K21, N24, S25, L27, N28 do neither affect the VIP activity nor its structure. Mutations are purely functional since they affect the biological activity with no structural change : H1, V5, R14, Y22, L23 and I26. Last, mutations of D3, F6, T7, D8, Y10, R12, L13, K15, K20 modify both the structure and the biological activity. In that last class, mutations decrease the activity to different extent. This means that some residues should be both functionally and structurally important, the part of each effect being not easily distinguished.

In conclusion, the molecular modeling using a recent « ab initio » procedure, OSIRIS, in combination with experimental analyses allows the distinction between structural (or structural/functional) and functional residues in a small peptide. This kind of approach should help to develop a comprehensive pharmacology for the VIP peptide.

References

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