CELLULOSE AS A MATRIX FOR SYNTHESIS OF THE LIBRARY OF MOLECULAR RECEPTORS USEFUL FOR SCREENING OF ANTIHISTAMINE COMPOUNDS

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Introduction

Cellulose is a biopolymer composed of β D-gluco-pyranose residues bonded with 1,4-glycosidic bonds. Its characteristic feature is the equatorial arrangement of secondary hydroxyl groups C2 and C3 and also the primary hydroxyl group C6. The result of this arrangement is the regular structure of cellulose resulting from the high content of crystalline phase [1]. In addition, the reactivity of particular hydroxyl groups is clearly defined. It is assumed that primary hydroxyl groups are about 25 times more reactive than secondary Presented facts make cellulose and its derivatives widely applicable [2]. It has also been shown that cellulose can be used as a membrane for bounding on its surface N-lipidated peptides which are able to mimic natural receptors and/or enzymes [3,4] by formation of binding cavities which are able to interact with different ligands recognizing their size, shape, charge distribution, chirality and polarity. Process of binding is reversible due to the nature of interactions between ligands and the binding pockets and it has been found that mechanism of binding is competitive and therefore the described process is mimicking the interactions involving natural receptors [5]. Herein we present an attempt to test whether a library of peptides immobilized on cellulose can mimic a histamine receptor and thus be used in studies with antihistamine active compounds. It is expected that it would be possible to select molecular receptors selectively interacting with agonists and antagonists.

Materials and Methods

Whatman-7 filter paper was used as matrix in the study. Cellulose was modified with 2,4-dichloro-6-methoxy-1,3,5-triazine and m-phenylenediamine according to standard protocol [5]. Syntheses of N-lipidated immobilized peptides were made by automated SPOT methods using as a coupling reagent DMT/NMM/TosO⁻. In all cases, two identical libraries were synthesized on each cellulose sheet. After splitting for two parts, one of them was treated with active substance and then with reporter dye (Brilliant Black), the second one was used for bounding the reporter dye only. For docking studies were used Histamine, Diphenhydramine, Doxylamine, Cimetidine, Ranitidine. All cellulose sheets after experiments were dried, scanned, and processed using Image-Quant program. Ability of molecular receptors to interact with colorless active compounds was calculated as difference in intensity of coloration by reporter dye and intensity of coloration after treatment with colorless ligand and subsequently with reporter dye. In this way, for each spot was determined average value of "gray" coloration calculated corresponding to interaction between binding pocket of molecular receptor and antihistamine ligand.

Results and Discussion

In order to study a representative number of peptide structures involved in the formation of molecular receptors were used modified SPOT methodology.

N-Lipidated peptides were immobilized on cellulose via aromatic linker containing fragments of m-phenylenediamine and 1,3,5-triazine connected with the cellulose surface in highly selective reaction with primary hydroxymethyl groups (FIG. 1).
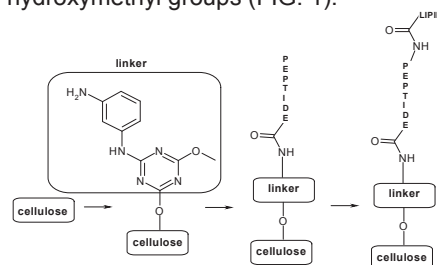

FIG. 1. Methods of synthesis of N-lipidated peptides immobilized on the cellulose.

In this studies were prepared the randomised library of N-heptanoylated dipeptides. As a C-terminal amino acids were used: alanine, proline and phenylalanine, as N-terminal residues were applied all natural amino acids. Finally, it was synthesized 60-elemets library of molecular receptors. As ligands for docking processes were applied compounds with a documented H1-H4 agonistic and antagonistic activity and Histamine as a natural ligand. As agonists were used Diphenhydramine and Doxylamine, as antagonists: Cimetidine and Ranitidine. The acquired results shown that binding pockets created by N-heptanoylated peptides are able to selective binding of tested anitihistamine compounds (FIG. 2).
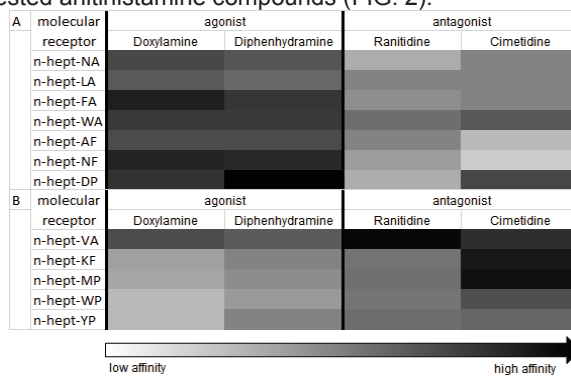

FIG. 2. Map of interaction between molecular receptors and tested compounds, A panel – strong bounding of agonists; B panel – strong bounding of antagonists.

Conclusions

These studies revealed that library of molecular receptors is capable in recognition and differentiation of agonistic/antagonistic profile of antihistamine active compounds. Even not understanding of complex relations between the structure of the molecular receptor and structure of the pharmacologically active substance, this should allow the construction of a new research tool useful as a platform for screening of new antihistamine compounds.

Acknowledgments

References

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