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SHORT COMMUNICATION

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Chromosomal localization of the human histamine H_1-receptor gene

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Abstract We have assigned the human histamine H_1-receptor gene to chromosome 3 by Southern blot analysis of a chromosome mapping panel constructed from human-hamster somatic cell hybrids. This assignment was confirmed by in situ hybridization on metaphase chromosomes and involved bands 3p14–p21.

Introduction

Histamine is an ubiquitous messenger molecule released from mast cells, enterochromaffin-like cells, and neurons. Its various actions are mediated by three pharmacologically defined receptors termed the H_1, H_2, and H_3 receptors (reviewed in Schwartz et al. 1991). The H_1 receptor was the first member of this family to be pharmacologically defined with the design of selective antagonists, the "antihistamines" (Bovet and Staub 1973), which are still currently used to treat allergic and inflammatory reactions.

The H_1 receptor appears to be mainly coupled in a positive manner with phospholipase C and, thereby, to elicit a variety of calcium-mediated responses (Hill and Donaldson 1992), including arachidonic acid release (Leurs et al. 1994). Its biochemical properties and tissue distribution have been studied with the help of reversible ligands such as [^{3}H]mepyramine (Hill et al. 1977) or [^{125}I]iodobolpyramine (Körner et al. 1986)and an irreversible photoaffinity ligand, [^{125}I]iodoazidophenpyramine (Ruat et al. 1988).

The H_1 receptor seems to be expressed by various peripheral tissues, such as smooth muscles, and by neurons in the brain, where histamine might be involved in the control of wakefulness, mood, and hormone secretion. Nevertheless, pathological conditions associated with changes in the expression or properties of H_1 receptors have never been clearly identified.

Recently, a bovine H_1-receptor cDNA was cloned, using a transfected oocyte as the expression system, and its nucleotide sequence was established (Yamashita et al. 1991). Its homology with the corresponding sequence of other receptors confirmed that it belongs to the superfamily of receptors coupled with G proteins with seven putative transmembrane domains. Subsequently, cDNAs of rat (Fujimoto et al. 1993) and guinea pig homologs (Traiffort et al. 1994) were cloned and, in the latter case, characterized in detail with regard to pharmacology and transduction systems (Leurs et al. 1994). As a prerequisite of genetic studies of the histamine H_1 receptor, we report here its human chromosomal localization.

Materials and methods

Localization by hybridization to DNA extracted from human-hamster somatic cell hybrids

DNA extracted from human-hamster somatic cell hybrids containing a reduced number of human chromosomes, digested with the *Eco*RI restriction enzyme, and blotted to a nylon membrane (Bios chromosome panel blot CB-1AI and CB-2BII, Bios Corporation) was used. The blots were prehybridized for 2 h at 65° C and, then, hybridized overnight at the same temperature in 6 × SSC, 5 × Denhardt's, 10% dextran sulfate, 1% SDS, 100 µg/ml denatured Salmon sperm DNA. The membranes were washed twice in 2 × SSC, 0.5% SDS for 10 min at room temperature, once in 1 × SSC, 1% SDS for 15 min at 65°C, and twice in 0.1% SSC, 1% SDS for 15 min at 65° C.

The hybridization probe was obtained by the polymerase chain reaction (PCR; Saiki et al. 1988). The pSVH$_1$ plasmid containing the nucleotide sequence corresponding to the guinea pig H_1-receptor gene (Traiffort et al. 1994) was used as a template. A 692-bp nucleotide fragment encoding the putative transmembrane domains I–V of the guinea pig H_1 receptor was amplified using 50 n*M* primer 1 (5′-AAGACAGGATGTTGGAGGGCAACA 3′) and 50 n*M* primer 2 (5′-CAACTTCAGCTTCATCTCTGAGAA 3′) for 35 identical cycles (94° C, 56° C, and 72° C for 1 min each) with 5 u Amplitaq DNA polymerase (Perkin Elmer). PCR products were electrophoresed on a 1.5% agarose gel. The sole DNA fragment

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generated was of the expected size ; it was excised and purified by electroelution (Maniatis et al. 1982). The ^{32}P-fragment was obtained by nick-translation and used as a hybridization probe (~3.5 $\times 10^6$ dpm/ml). The blots were then exposed to X-ray films at –80° C for 24 h with two intensifying screens.

Localization by in situ hybridization

In situ hybridization experiments were performed using the same probe as that prepared for Southern blot analyses, but it was tritium-labeled with the multiprime DNA labeling system (Kit Amersham, UK). Its specific activity was 6.3×10^7 cpm/µg. In situ hybridization of the probe (concentrations of 20, 50, and 80 ng/ml) to normal human metaphase chromosomes was performed as previously described (Le Coniat et al. 1989).

Results

The chromosomal localization of the human histamine H_1-receptor gene was first determined by Southern blot analysis of two panels of 18 human-hamster cell hybrids. The ^{32}P-radiolabeled DNA fragment encoding transmembrane domains I – V of the guinea pig histamine H_1-receptor gene hybridized with human and hamster *Eco*RI genomic DNA fragments of 12 and 18 kb, respectively. No other signal appeared after a prolonged exposure time of one week. In each panel, only four of the 18 hybrid cell lines (numbers 423, 860, 507, 1079, as described by the purchaser; Smith et al. 1988) gave a positive hybridization signal corresponding to the presence of the human reactive *Eco*RI DNA fragment of 12 kb (data not shown). These four hybrid cell lines being the only ones to contain the human chromosome 3, a perfect match was thus observed between the signal and the presence of chromosome 3 in both panels.

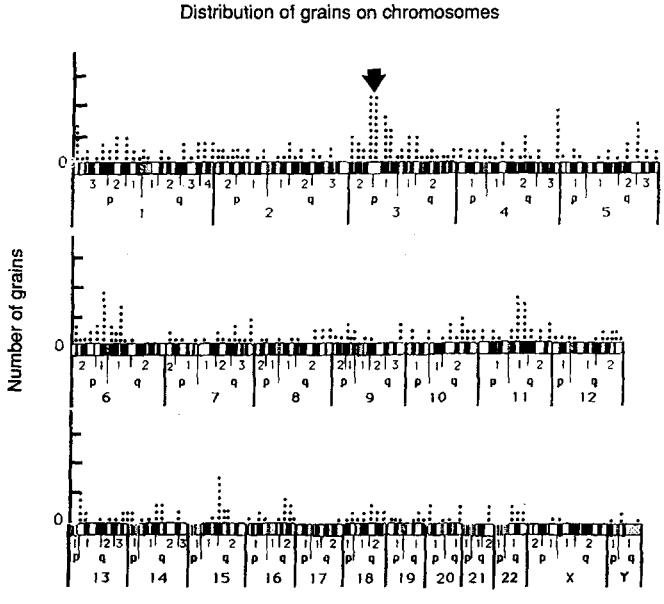

Fig. 1 Distribution of silver grains on 256 metaphase cells after in situ hybridization with the H_1 receptor gene probe. *Arrow* localization of the probe

Two in situ hybridization experiments were performed with identical results, and so they could be pooled. A total of 399 silver grains were counted on 256 metaphase cells (Fig. 1); 59 (14.8%) were on chromosome 3 of which 27 (6.8% of the total number) were localized to 3p14–p21. Accumulations of grains were observed on other bands but with lower percentages, viz., 4q35 (2% of the total), 6p11 (2%), 11q13–q14 (3.3%), 15q21(1.75%). It was concluded that the most probable localization of the histamine H_1-receptor gene was 3p14-p21.

Discussion

The histamine H_1-receptor gene has been assigned to chromosome 3 by somatic cell hybrid studies, and localized to bands 3p14–p21 by in situ hybridization experiments. The background, similar in the two in situ hybridization experiments performed, may be attributable to the differences between species, since the probe was prepared from the guinea pig DNA sequence of the gene. Another possible explanation for the presence of the minor peaks of accumulation of grains (Fig. 1) is that the H_1-receptor gene belongs to a family of genes that may have sufficient homology to hybridize partially with the probe used in these in situ experiments. With this technique, the conditions of stringency are not as easy to control as in Southern blotting experiments. Nevertheless, the specificity of the signal on chromosome 3 was evident after a prolonged exposure time of the hybridized panels of human-hamster cell hybrids, since no other signal was observed.

The present localization of the histamine H_1-receptor gene is indeed the first localization of one histamine receptor gene to human chromosomes, but other receptors have been defined (Schwartz et al. 1991). It will be interesting to determine whether the genes encoding the other histamine receptors are clustered.

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