neuroReport

NeuroReport 7, 1293–1296 (1996)

Allelic variations of the human histamine H_2 receptor gene

Paul R. Orange, Paul R. Heath, Simon R. Wright and R. Carl A. Pearson[CA]

Department of Biomedical Science, University of Sheffield, Sheffield S10 2TN, UK

[CA]Corresponding Author

THE gene encoding the human histamine H_2 receptor (H2R) has previously been cloned and sequenced from gastric cDNA. Following PCR amplification of a fragment of the H2R gene from total human DNA, three single nucleotide base substitutions were observed and confirmed when compared with the previously published sequence. One of these base changes introduces an additional *Taq*I restriction endonuclease site in the coding portion of the gene. PCR amplification of human H2R gene fragments followed by cleavage with *Taq*I demonstrated the existence of allelic variation of the human H2R gene.

Key words: Monoamine; DNA; Gene sequence; Schizophrenia; Parkinson's disease; Alzheimer's disease; PCR

Introduction

Histamine is active in many systems and organs in the body, including the gastrointestinal tract, the immune system and the brain.[1] This monoamine signal transmitter acts via three identified cell surface receptors, the histamine H_1, H_2 and H_3 subtypes. Histamine is a neurotransmitter in the central nervous system (CNS) and is important in the regulation of sleep/wake cycles.[3] Within the CNS, histaminergic axons originate predominantely in the tuberomamillary nucleus of the posterior hypothalamus, from where they distribute widely, including to the cerebral cortex.[4] Post-synaptic histamine actions in the CNS are mediated via the H_1 and H_2 receptors, with the H_3 receptor acting as an autoreceptor regulating histamine release.[5] The potential significance of histamine and its post-synaptic receptor in human neurological and neuropsychiatric disease has been highlighted recently by reports of the efficacy of a specific histamine H_2 receptor (H2R) antagonist, Famotidine, in the treatment of some schizophrenic symptoms[6–9] and Parkinson's disease.[10] A further report suggests that H2R antagonists may be useful therapeutic agents in Alzheimer's disease.[11] Given the reported associations between allelic variation in similar aminergic receptors and treatment efficacy in neuropsychiatric disease,[12–15] it was decided to investigate the existence of sequence variability in the human H2R gene.

Materials and Methods

All materials were obtained from Sigma (UK) unless otherwise indicated. All waste products containing human material and/or genetically modified organisms were disposed of in accordance with local regulations.

Production and sequencing of a 1045 base pair fragment of the human H2R: Total human DNA was extracted from post-mortem human brain tissue using a standard phenol–chloroform extraction technique.[16] DNA (0.5 μg) was amplified by polymerase chain reaction (PCR), for 36 cycles. The timing for each cycle was as follows: 60 s at 94° C, 90 s at 56° C and 120 s at 72° C. This was then followed by 10 min at 72° C. The reaction conditions were: 3 mM magnesium chloride, 1 × PCR buffer, 20 nmol of each deoxynucleotide triphosphate (dNTP), 2 units Taq DNA polymerase (Perkin-Elmer) and 20 pmol of each primer. The volume of the reaction mix was then adjusted to 100 μl with sterile DNAase free water. The oligonucleotide primers used were, 5′ CCAATGGCACAGCCTCTT 3′ and 5′ CGTGACTTCTGTCCCACT 3′, corresponding to bases 8–25 and 1036–1053, respectively, of the human gastric cDNA sequence.[17] This reaction produced a single DNA fragment of 1045 base pairs when analysed by gel electrophoresis. Following PCR amplification the resulting DNA fragment was immediately ligated and cloned into the TA cloning system (InvitroGen). Positively transformed colonies were analysed by growing a 5 ml Luria Bertani culture overnight at 37° C, extracting the plasmids (Qiaspin minipreps [Qiagen]) and performing an *Eco*RI restriction nuclease (Amersham) digest to ensure the correct sized product was contained in the plasmid. This plasmid was then nominated PO1. Both strands of the cloned PCR product were sequenced using the dideoxynucleotide chain-termination method, carried out with Sequenase version 2.0 (Amersham).

Analysis of H2R allele: A 10 ml sample of blood was collected from healthy volunteers into ethylenediaminetetraacetic acid (EDTA) lined tubes (Vacutainer). DNA was extracted from these samples by heating 1 ml of blood to 100°C for 15 min. This was then centrifuged at 13 000 r.p.m. for 15 min in a microcentrifuge (MSE). The supernatant was kept for use as template DNA in subsequent PCR reactions and the cell pellet discarded. DNA was also extracted from the brain tissue of neurologically and neuropsychriatrically normal controls using a standard phenol chloroform extraction technique.[16] To assess these cases for the presence of the new allele of the H2R gene (as present in the plasmid PO1), 1 μl of the extracted plasma or 0.5 μg of DNA extracted from brain tissue was amplified by PCR for 35 cycles. The timing of each cycle was 60 s at 96°C, 60 s at 56°C, 80 s at 72°C. The reaction conditions were: 3 mM magnesium chloride, 10 nmol of each dNTP, 1× PCR buffer, 1 unit Taq DNA polymerase (Gibco BRL) and 10 pmol of each primer. The volume of the reaction mix was then made up to 50 μl with sterile, DNAase free water. The oligonucleotide primers used were 5′ CCAATGGCACAGCCTCTT 3′ and 5′ GTTGTCGAGAAGACGACG 3′, corresponding to bases 8–25 and 898-915, respectively, of the gastric cDNA sequence of the H2R.[17] Following amplification, 10 μl of the reaction mix was analysed by gel electrophoresis to ensure correct amplification of a DNA fragment 909 base pairs long. PCR product (11.5 μl) was then added to 20 units of *Taq*I restriction endonuclease (Gibco BRL) and the appropriate reaction buffer and incubated at 65°C for 18 h. The products of the reaction were then analysed by separation on a 3% agarose gel (see Fig. 1). To ensure no cross-contamination of the PCR reactions, a negative control consisting of all the reaction components from a 'master mix' except the template DNA was included. Also included as a positive control was a PCR reaction utilizing the plasmid PO1 as template DNA. This positive control assured that complete cleavage of the PCR products occurred.

Direct sequencing of PCR products: DNA (0.5 μg) extracted from the brain of five separate cases was amplified by PCR for 35 cycles. The timing for each cycle was as follows: 60 s at 96°C, 90 s at 56°C and 120 s at 72°C. The reaction conditions were: 3 mM magnesium chloride, 1 × PCR buffer, 10 nmol of each deoxynucleotide triphosphate (dNTP), 1 unit Taq DNA polymerase (Gibco BRL) and 10 pmol each primer. The volume of the reaction mix was then adjusted to 50 μl with sterile DNAase free water. The oligonucleotide primers used were 5′ ACACCAGCCTGGATGTGA 3′ and 5′ GTTGTCGAGAAGACGACG 3′, corresponding to bases 281–398 and 898–915, respectively, of the human gastric cDNA sequence.[17] This reaction produced a single DNA fragment of 633 base pairs when analysed by gel electrophoresis. The PCR mix was then passed through a ProbeQuant™ G-50 micro column (Pharmacia Biotech) to purify out any residual primers, buffer or dNTP. One strand of each PCR product was sequenced using the dideoxynucleotide chain-termination method, carried out with Sequenase version 2.0 (Amersham).

Results

When sequenced, the 1045 base pair PCR product contained in the plasmid PO1 was homologous to the published H2R sequence,[17] except for the substitution of a guanidine (G) for an adenosine (A) at base 649. Five additional base substitutions were also seen on sequencing of this cloned PCR product, at bases 398, 525, 620, 692 and 802 (Table 1). That the base change at position 649 was not a PCR artifact was confirmed by direct sequencing of further H2R gene PCR fragments from another five individuals, three of whom displayed the base substitution at base 649 (two in a heterozygous fashion and one in a homozygous fashion). Two of the other base substitutions (at positions 692 and 802) were also seen on direct sequencing of these H2R PCR fragments (as above) and were heterozygous or homozygous in accordance with the substitution at base 649. In the two other cases the base substitution at position 649 was not seen and neither were any of the other base substitutions described in the plasmid PO1. These data suggest that there is co-segregation of the base substitutions at positions 649, 692 and 802. When these PCR products were analysed by *Taq*I restriction endonuclease digestion (see below), they cleaved in a manner predicted by their sequence. The A to G base change at position 649 introduces an additional *Taq*I restriction site in the coding portion of the H2R gene. Digestion of a 909 base pair PCR fragment of the H2R gene (corresponding to bases 8–915) containing this base change generated DNA fragments of 335, 306 and 268 base pairs, distinct from the DNA fragments of 574 and 335 base pairs seen following digestion of the same PCR fragment lacking this base change (i.e. the originally described sequence[17]). If both of these forms of the H2R gene

Table 1. Base changes seen in the 1045 base pair H2R PCR fragment contained in the plasmid PO1

Site of base change	Base	Resultant amino acid alteration
398	T→C	Valine→Glycine
525	A→T	Lysine→Asparagine
620	A→G	Lysine→Aspartate
649	A→G	Asparagine→Aspartate
692	A→G	Lysine→Arginine
802	G→A	Valine→Methionine

FIG. 1. *Taq*I restriction endonuclease digestion of a 909 base pair PCR fragment of the human histamine H2 receptor gene, separated by electrophoresis on a 3% Tris-Borate-EDTA agarose gel, stained with ethidium bromide. **Lane A**: DNA band pattern indicative of a H2R649A homozygote; **Lane B**: DNA band pattern indicative of H2/R649A/H2R649G heterozygote; **Lane C**: DNA band pattern indicative of a H2R649G homozygote. Numbers at the right hand side indicate the DNA fragments sizes seen in lanes A–C.

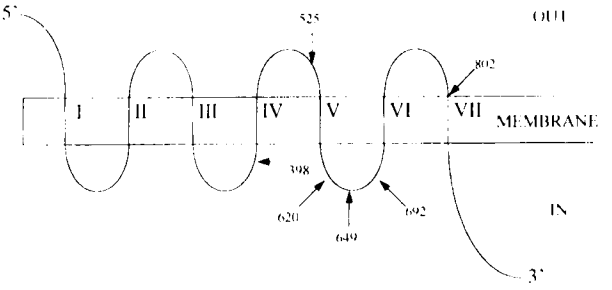

FIG. 2. The positions of the six base substitutions (Table 1) in relation to the postulated structure of the receptor protein.

were present in a given sample, then *Taq*I digestion would reveal DNA fragments of 574, 335, 306 and 268 base pairs. The original sequence[17] was nominated H2R649A; the new sequence containing the base substitution at position 649 as H2R649G. To examine the occurrence of the base substitution at position 649 in a wider population, and to further prove its existence, DNA was extracted from the blood of healthy volunteers, or post-mortem brain tissue of neurologically normal controls. These DNA samples were then amplified by PCR to produce a 909 base pair H2R fragment (as above) which was digested with *Taq*I restriction endonuclease. DNA fragments corresponding to H2R649A/H2R649G heterozygotes, H2R649A homozygotes and H2R649A homozygotes were seen (Fig. 1). The incidence of the genotypes was 34.5% H2R649A/H2R649A, 43.1% H2R649A/H2R649G, 22.4% H2R649G/H2R649G in a population of 58 samples.

Discussion

Allelic variation in the genes encoding aminergic neurotransmitter receptors associated with neuropsychiatric diagnoses, disease susceptibility and treatment efficacy has been extensively investigated.[18–20] The present data demonstrate at least one allelic variation in another human aminergic receptor gene, a base substitution at base 649 of the human H2R, of an A for a G. There is also evidence of additional base substitutions in the gene, which require further elucidation and confirmation. These additional base changes (at positions 398, 525, 620 and 802) could either indicate that the allelic variant H2R649G is different at multiple sites from the published sequence,[17] or that there exists a family of allelic variants of the H2R gene. This requires further investigation. However, the possibility that the base changes at positions 398, 525, and 620 are PCR artifacts, cannot be discounted given the present data.

The demonstrated base substitution and all of the other possible sequence changes lead to changes in the proposed amino acid sequence of the receptor, some of which are non-conservative in nature. Three of these, including the allelic variant demonstrated here, lead to changes in the amino acid sequence of the postulated third intracellular loop of the receptor protein (Fig. 2). Given the important role of this domain in the interaction of the receptor with G-protein mediated intracellular second messenger systems,[21] these base changes have potentially major implications for the functioning of the encoded receptor. It is also important to note that none of the changes introduce a stop codon into the gene, which would potentially produce a truncated form of the protein.

Given the observations that H2R antagonists have beneficial effects in human neurological and neuropsychiatric conditions such as Alzheimer's disease,[11] Parkinson's disease[10] and schizophrenia,[6–9] these data have potentially major implications. Histaminergic involvement in diseases such as these, may, in part, be attributable to differing second messenger activation of different forms of the histamine H2 receptor.

These data also prompt further work, specifically examining the possible coincidence of allelic variation of the gene in relation to neurological disorders, including schizophrenia, Parkinson's disease and Alzheimer's disease. Further priorities include expression of the allelic variants to examine the potentially different functioning of the proteins that they encode.

Conclusion

This is the first time that allelic variation in the human H2R has been demonstrated. This variation consists of three single base changes which appear to co-segregate with each other, the presence of which has been demonstrated in a population of 58, as well as three other possible base substitutions.

References

1. Parsons ME. *Scand J Gastroenterol* **26**, 46–52 (1991).
2. Atrang J-M. *Cell Mol Biol* **40**, 273–279 (1994).
3. Lin JS, Sakai K and Jouvert M. *Eur J Neurosci* **6**, 618–625 (1994).
4. Onodera K, Yarmadotani A, Wantenabe T *et al. Prog Neurobiol* **42**, 685–702 (1994).
5. Arrang J-M, Garoarg M and Schwartz J-C. *Neuroscience* **15**, 533–562 (1985).
6. Kaminski R, Moriarty TM, Bodine J *et al. Lancet* **335**, 1351–1352 (1990).
7. Rosse RB, Kendrick KA, Faymccarthy M *et al. Schizophrenia Res* **15**, 148 (1995).
8. Deutsch SI, Rosse RB, Kendrick KA *et al. Clin Neuropharmacol* **16**, 518–524 (1993).
9. Rosse RB, Kendrick KA, Tsui LC *et al. Clin Neuropharmacol* **18**, 369–374 (1995).
10. Molrnari SP, Kaminski R, Dirocco A, *et al. J Neural Transm Parkinson's Disease and Dementia Section* **9**, 243–247 (1995).
11. Breitner JCS, Welsh KA, Helms MJ *et al. Neurobiol Aging* **16**, 523–530 (1995).
12. Shaikh S, Collier D, Gill M *et al. Schizophrenia Res* **11**, 146 (1994).
13. Sodhi M, Arranzcalderon MJ, Collier D *et al. Schizophrenia Res* **15**, 49–50 (1995).
14. Atranz MJ, Aitchinson KJ, Kerwin R *et al. Schizophrenia Res* **15**, 35 (1995).
15. Arranz M, Collier D, Sodhi M *et al. Lancet* **346**, 281–282 (1995).
16. Sambrook J, Fritsch EF and Maniatis T. In: Sambrook J, Fritsch EF and Maniatis T, eds. *Molecular Cloning, A Laboratory Manual, 2nd edn.* Coldspring Harbor Press, 1989: 9. 16–9.11.
17. Gantz I, Munzert G, Tashiro T. *et al. Biochem Biophys Res Commun* **178**, 1386–1392 (1991).
18. Shaikh S, Collier D and Kerwin R. *Schizophrenia Res* **15**, 48 (1995).
19. Sarkar G, Kapelner S, Grandy DK *et al. Genomics* **11**, 8–14 (1991).
20. Daniels J, Williams R, Mant P *et al. Am J Med Gen* **54**, 256–258 (1994).
21. Leurs R, Smit MJ, Menge WMBP *et al. Br J Pharmacol* **112**, 847–854 (1994).

ACKNOWLEDGEMENTS: This work was kindly supported by the schizophrenia research fund UK and the Mental Health Foundation. We would also like to acknowledge the help of Victoria Pearson.

Received 20 December 1995; resubmitted 21 February 1996; accepted 19 March 1996

General Summary

Differences in the genetic code between individuals for specific receptor molecules has been described as influencing susceptibility to neuropsychiatric disease, particularly schizophrenia, and responsiveness of individuals to treatment. This paper describes another such variation in a different receptor gene, one for histamine, and describes the incidence of two different sequences for this receptor in the normal population. Involvement of this histamine transmitter system in neuropsychiatric diseases, including schizophrenia, has been described, and the influence of this new sequence variation on the occurrence and course of these diseases requires investigation.