SUBSTITUTION OF TRYPTOPHAN WITH ALANINE IN HUMAN D1 DOPAMINE RECEPTORS

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Introduction[1,2]

The key brain neurotransmitter that is involved with learning, memory, pleasure, movement and proper cognition is dopamine. It has been long understood that the improper regulation of dopamine results in complications that can lead to the development of schizophrenia, parkinson's, and drug addiction. Dopamine exerts its affects by binding to ligand membrane receptors that belong to either the hD1 or hD5 family. These receptors are transmembrane receptors with seven protein domains and three intracellular loops. The purpose of this investigation is to examine the role of certain amino acids containing hydrogen donating groups in hD1 to learn about how they interact inside the cell, and whether one can alter the function of hD1 by simply substituting these amino acids with other amino acids with a less capability to hydrogen bond. In other words, missense mutations are induced to substitute certain amino acids in the hD1 receptor and then the mutated hD1 receptors are expressed to monitor their proper functioning. The amino acid of interest in this project is tryptophan (in position 148 of protein), an indole containing amino acid that can hydrogen bond by donating hydrogens. It will be substituted with alanine, an non-indole amino acid with a lower hydrogen bonding capability. In the past, many experiments have been conducted to explain the role of intracellular loop three (IL3) in the functioning of hD1 and hD5, but still not enough research has shed light onto this subject. Interestingly, not much is known about how dopamine receptors function. Hopefully, this investigation will enlighten current knowledge about dopamine receptors and will allow further investigations to develop a complete three dimensional model of these receptors. Having a 3D model allows for the development of specific therapeutic drugs that can potentially treat the neurological disorders mentioned above with minimal side effects.

Methods

A molecular biology approach was taken to create the desired amino acid substitution.

- First, PCR amplifications were used to generate megaprimers and the overlap
- Second, a DNA digestion was carried out using the enzymes EcoRI and HindIII to isolate the desired cassette
- Third, DNA ligation was conducted to link the cassette to the vector
- Fourth, XL1-blue bacteria were transformed with the newly made vector through electroporation
- Finally, bacterial colonies were isolated and a DNA extract was collected for sequencing

View figures 1 and 2.

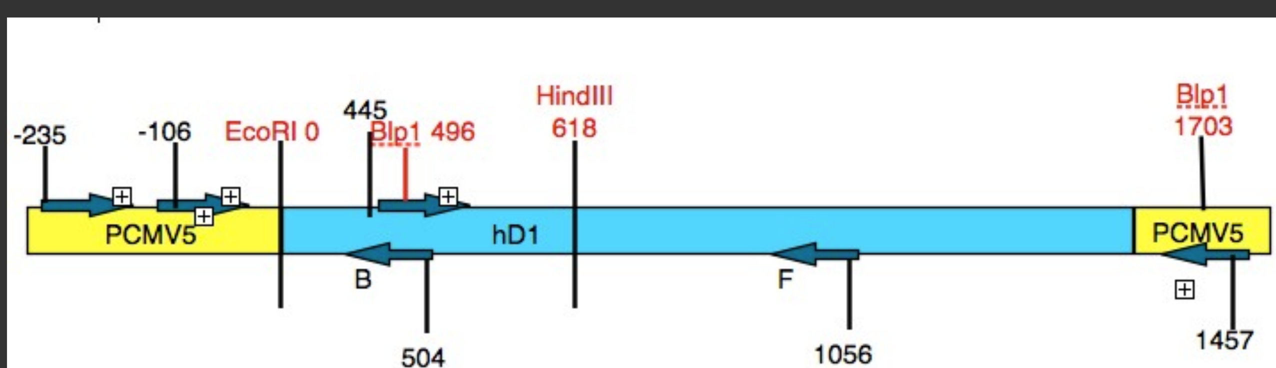

Figure 1: hD1 gene showing the binding sites of primers and enzyme cut sites for generation of the megaprimers and then the overlap.

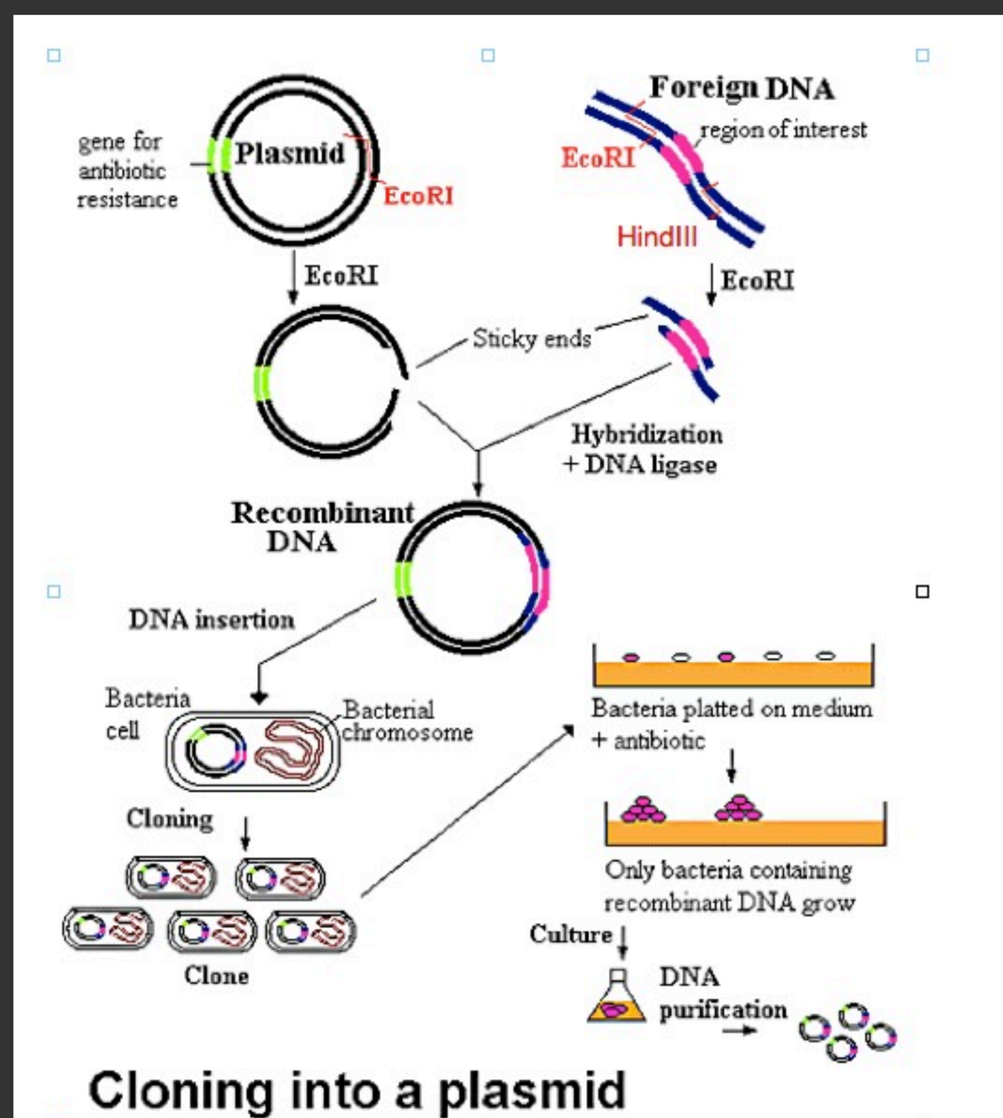

Figure 2[3]: The insertion of a desired sequence into a plasmid and then the transformation of the plasmid into the XL1-blue bacteria.

Results

Figure 3: Electrophoresis gel showing megaprimers AB and CD

Figure 4: Electrophoresis image showing DNA overlap.

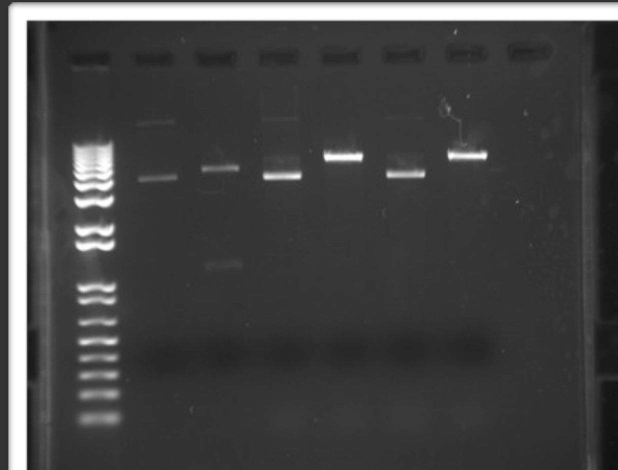

Figure 5: Electrophoresis image showing the casette.

hD1-W148A

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tacggtgggaggtctatataagcagagctcgtttagtgaaccgtcagaattcggatccaagATG
AGGACTCTGAACACCTCTGCCATGGACGGGACTGGGCTGGTGGTGGAGAGGGACTTCTCTGTTC
GTATCCTCACTGCCTGTTTCCTGTCGCTGCTCATCCTGTCCACGCTCCTGGGGAACACGCTGGT
CTGTGCTGCCGTTATCAGGTTCCGACACCTGCGGTCCAAGGTGACCAACTTCTTTGTCATCTCC
TTGGCTGTGTCAGATCTCTTGGTGGCCGTCCTGGTCATGCCCTGGAAGGCAGTGGCTGAGATTG
CTGGCTTCTGGCCCTTTGGGTCCTTCTGTAACATCTGGGTGGCCTTTGACATCATGTGCTCCAC
TGCATCCATCCTCAACCTCTGTGTGATCAGCGTGGACAGGTATTGGGCTATCTCCAGCCCTTTC
CGGTATGAGAGAAAGATGACCCCCAAGGCAGCCTTCATCCTGATCAGTGTGGCAGCGACCTTGT
CTGTACTCATCTCCTTCATCCCAGTGCAACTCAGCTGGCACAAGGCAAAACCCACAAGCCCCTC
TGATGGAAATGCCACTTCCCTGGCTGAGACCATAGACAACTGTGACTCCAGCCTCAGCAGGACA
TATGCCATCTCATCCTCTGTAATAAGCTTTTACATCCCTGTGGCCATCATGATTGTCACCTACA
CCAGGATCTACAGGATTGCTCAGAAACAAATACGGCGCATTGCGGCCTTGGAGAGGGCAGCAGT
CCACGCCAAGAATTGCCAGACCACCACAGGTAATGGAAAGCCTGTCGAATGTTCTCAACCGGAA
AGTTCTTTTAAGATGTCCTTCAAAAGAGAAACTAAAGTCCTGAAGACTCTGTCGGTGATCATGG
GTGTGTTTGTGTGCTGTTGGCTACCTTTCTTCATCTTGAACTGCATTTTGCCCTTCTGTGGGTC
TGGGGAGACGCAGCCCTTCTGCATTGATTCCAACACCTTTGACGTGTTTGTGTGGTTTGGGTGG
GCTAATTCATCCTTGAACCCCATCATTTATGCCTTTAATGCTGATTTTCGGAAGGCATTTTCAA
CCCTCTTAGGATGCTACAGACTTTGCCCTGCGACGAATAATGCCATAGAGACGGTGAGTATCAA
TAACAATGGGGCCGCGATGTTTTCCAGCCATCATGAGCCACGAGGCTCCATCTCCAAGGAGTGC
AATCTGGTTTACCTGATCCCACATGCTGTGGGCTCCTCTGAGGACCTGAAAAAGGAGGAGGCAG
CTGGCATCGCCAGACCCTTGGAGAAGCTGTCCCCAGCCCTATCGGTCATATTGGACTATGACAC
TGACGTCTCTCTGGAGAAGATCCAACCCATCACAAAACGGTCAGCACCCAACCTGAggatcc
tctagaggatcccgggtggcatccctgtgacccctccccagtgcctctcctggccctggaagtt
gccactccagtgcccaccagccttgtcctaataaaattaa
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Legend:
Blue = enzyme sequence cuting the cassette
Red = Mutations for generating alanine
Orange = Silent mutation used for screening

Figure 6[4]: DNA Sequence of hD1 gene showing missense mutation

Upon sequencing the DNA in the bacterial extract, the desired missense mutation was found. Bacterial colonies had grown on a medium containing nutrients and an antibiotic.
Figures 3,4, and 5 show the gel electrophoresis of the megaprimers, the overlap, and the cassette respectively. Figure 6 shows the DNA sequence of the cassette after it was isolated from the bacterial colonies along with a legend. Figure 3 indicates that there is a megaprimer around 739 base pairs (bp) and another megaprimer around 1012 bp. Figure 4 shows the overlap product at around 1162 bp. Finally, figure 6 shows the DNA sequence of the cassette and a GC base mutation within that sequence.

Conclusion

In conclusion, the hD1 gene has been mutated to generate alanine instead of tryptophan in the hD1 human receptor. The DNA sequence obtained from the bacterial extract shows that the bases G and C were incorporated in the DNA. The mutated gene can now be inserted in human basal cells where the dopamine receptor will be expressed. Further investigations will be able to measure the functioning of the receptor and how it interacts with drugs, hopefully shedding light about the structural and functional characteristics of the different regions of hD1 receptor.

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Bibliography

1. Missale, Cristina, Russel Nash, Susan W. Robinson, Mohamed Jaber, and Marc G. Caron. "Dopamine Receptors: From Structure to Function." *Physiological Revies* 78.1 (1998): 189-225. Abstract. (n.d.): n. pag. Print.
2. "Alanine to Tryptophan." *Alanine to Tryptophan.* N.p., n.d. Web. 15 Jan. 2013. <http://www.biology.arizona.edu/biochemistry/problem_sets/aa/Anim/ala_trp.html>.
3. http://www.accessexcellence.org/RC/VL/GG/images/PLASMID.gif
4. Boyang Zhang. PHD. in progress

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