REPORT

Major Case study

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Biochemistry II

Course code: BSBT 530

STRUCTURAL ANALYSIS OF LIGAND-RECEPTOR AND DRUG-RECEPTOR COMPLEXES BY VIRTUAL MOLECULAR DOCKING AND VISUALIZATION

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Contents

Abstract

Prolactin hormones stimulate the biosynthesis or production of mother milk by the lactase enzyme and Oxytocin hormone stimulate contraction in the uterine site or also in mesothelium cell of the alveoli of the breast[1]. Prolactin is a main hormone which is responsible for the growth as well as differentiation of the mammary glands. Prolactin is seen to be quite active during pregnancy and is on the alert for new adaptations that are taking place inside the body. Without oxytocin hormone women are not able to balance their reproductive tract[1]-[2]. At time of baby birth or labor time of women Oxytocin secrete from hypothalamus, release into blood and transformed through chemical signaling towards the uterine site for the uterus contraction. At time of baby birth or labor time of women Oxytocin secrete from hypothalamus, release into blood and transformed through chemical signaling towards the uterine site for the uterus contraction[3]. Oxytocin hormone are non peptiditic/natural synthesis hormone which have nine pairs long in length with one disulfide linkage at 1-6 residue. Oxytocin one of the best-known hormones that helps in both site of the women's, in breast it helps in milk feeding and in reproductive system it helps in blood clotting at time labor or also makes contraction in the uterus during the birth of child. Women's who were suffering from PCOS and who have not conceived yet, the levels of prolactin were seen inversely linked with some harmful metabolic indicators like, triglyceride, total cholesterol circumference of the waist etc[4]. A lot of changes are found in glucose digestion, expanding peripheral insulin opposition in pregnancy and lactation period, initiating with beta cell mass compression, presumably even apoptosis, and diminished insulin discharge. During the breastfeeding period insulin have an insulinotropic effect[4]. Physiologically raised prolactin may protect from the impedance of glucose homeostasis though incredibly significant levels of prolactin massively increases resistance of insulin and harm insulin-secretory limit in certain circumstances, for example,prolactinoma[4]-[5].

Keywords

Mesothelium cell, PCOS, Lactogenesis, Prolactin & Oxytocin, Estrogen & Progesterone, Autoimmune diseases, Proliferation, Poor metabolism, Osmoregulation, Betatrophin

Introduction

There are two types of primary hormones which are necessary for lactation including Oxytocin and Prolactin hormone. Prolactin hormones stimulate the biosynthesis or production of mother milk by the lactase enzyme and Oxytocin hormone stimulate contraction in the uterine site or also in mesothelium cell of the alveoli of the breast[6]. According to pharmacological site or level of estrogen hormone (either it will be exogenous source or endogenous source) can be stop or block the Prolactin activity. At time of another pregnancy Progesterone stop biosynthesis of Prolactin hormone. Prolactin linked with breast feeding as well as metabolism v/s the role of Prolactin in PCOS. The main hormone which is responsible for the growth as well as differentiation of the mammary glands is Prolactin. In the sequential manner the process of the lactogenesis is also dependent on this hormone. This hormone is not only secreted by the pituitary gland but can also be found in the lining of the adipose tissue present inside the macrophages which in turn are responsible for inflammatory response and in maintaining elevated concentrations of Glucose[7].Some cross-sectional studies, in recent years have found that the higher the level of prolactin the more increased was the seen in the number of white blood cells and which also increased the risk of autoimmune disease].Prolactin is seen to be quite active during pregnancy and is on the alert for new adaptations that are taking place inside the body. This hormone helps to build resistance against insulin during pregnancy by stimulating the proliferation of the β-cell present in the islets of pancreas[8]. In women who were suffering from PCOS and who have not conceived yet, the levels of prolactin were seen inversely linked with some harmful metabolic indicators like, triglyceride, total cholesterol circumference of the waist etc. Insufficient increase of breast at the time of pregnancy is also associated with the poor metabolism at the time of pregnancy (in both early and late phase) when compared with increase in breast between women diagnosed with PCOS. Studies have revealed that the obese

women and women suffering from PCOS have shorter breastfeeding duration as compared to healthy lactating women with a normal weight[9]. Therefore, obesity could be one of the prominent factors that contributes and probably is responsible factor among women having PCOS who suffer with shorter breastfeeding problem. Prolactin has various other significant roles in different situations like osmoregulation, immune regulation but our focus is on reproduction metabolic[10]. Due to post translational modifications prolactin have many different isoforms with specific biological activities to perform. Generally, there is an inverse relation between the prolactin and glucose levels the higher the prolactin, lower will be the glucose level, lipid levels and higher will be the insulin sensitivity. During the breastfeeding period insulin have an insulinotropic effect. Physiologically raised prolactin may protect from the impedance of glucose homeostasis though incredibly significant levels of prolactin massively increases resistance of insulin and harm insulin-secretory limit in certain circumstances, for example, prolactinoma. Patients having prolactinoma have hyperglycemia, are over-weight, and become insulin resistant[11]. Prolactin organises insulin affectability, glucose digestion, improving hepatic insulin affectability, and helps to manage the immune function. Studies have showed inverse relation of prolactin with HbA1c and hour C peptide. A few investigations in the past showed that higher prolactin levels had critically lower prevalence of type 2 diabetes. Few studies show the present circumstance. The negative correlation between Hba1c, C peptide and prolactin may show the ascension in peripheral insulin affectability. As far as anyone is concerned this was the first run through to show the relationship among prolactin and fringe insulin affectability[2]. A lot of changes are found in glucose digestion, expanding peripheral insulin opposition in pregnancy and lactation period, initiating with beta cell mass compression, presumably even apoptosis, and diminished insulin discharge. There are a few reports showing that deliberate or circling elements can mastermind beta cell replication and mass[12,13]. A few chemicals like insulin, placental lactogen and prolactin have a part in directing beta cell mass. Betatrophin is a chemical that controls pancreatic beta cell multiplication. Betatrophin organizes lipoprotein lipase action and assumes huge parts in lipid and unsaturated fat digestion. Subsequently, prolactin can partake in the plan of glucose and lipid digestion comparable as betatrophin. Therefore, this relationship may affect breastfeeding[4-14].

Interconnection of women's reproductive hormones

Human reproductive hormones are interconnected to each other's by the process of hypothalamus stimulation level. Like in female Prolactin and Oxytocin hormone secretion depends on its level of expression if Prolactin secrete from anterior pituitary after the delivery of baby the serum progesterone level will be decreases, because of FSH and LH also change their expression level. When Prolactin hormone level increased it can be stop or suppress the level of Luteinizing hormone and Follicle Stimulating hormone and cause amenorrhea[12,15].

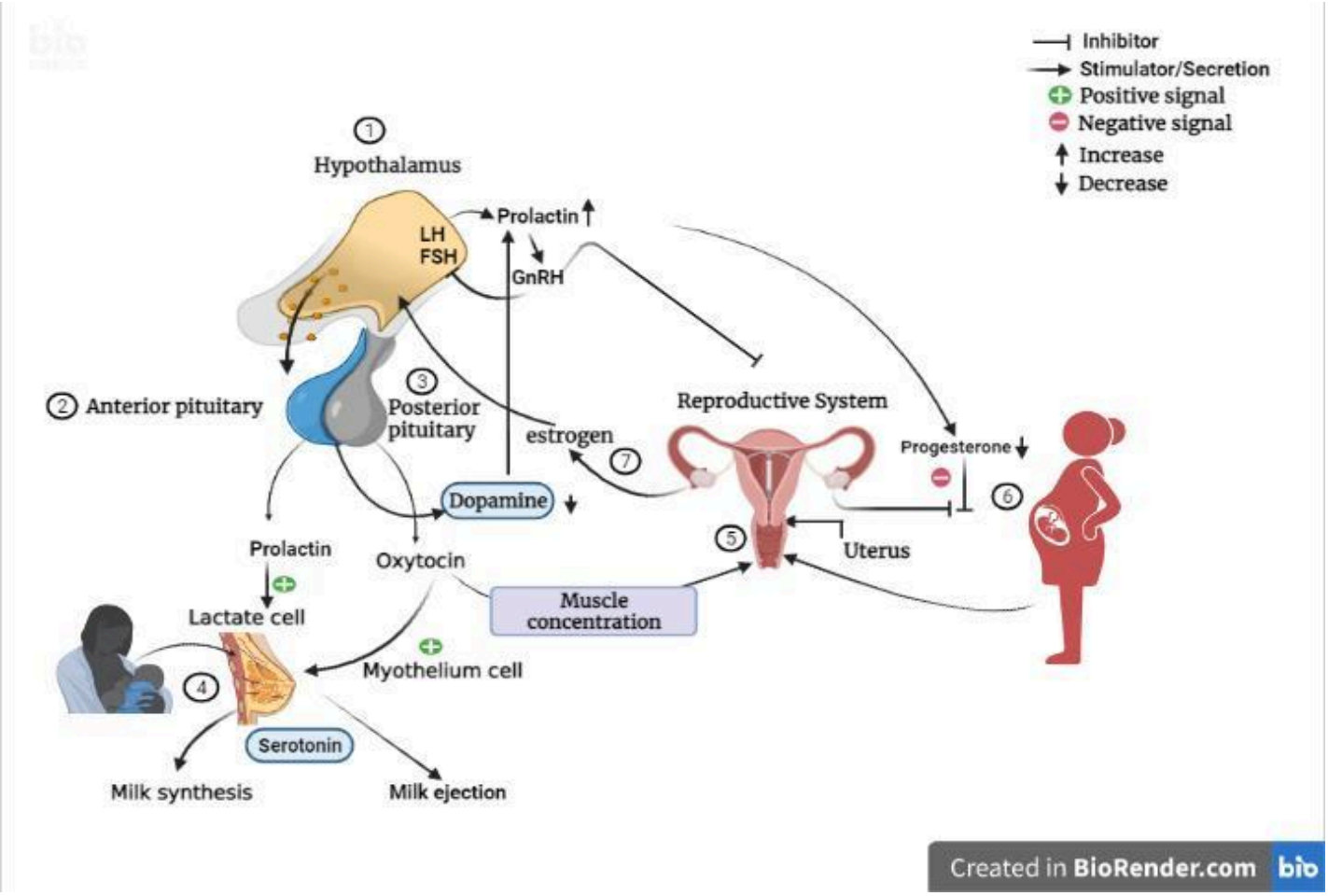


Fig.1: This whole figureillustration is created by BioRender http://:app.biorender.com/illustrations/edit/60a5ed7f33c8de00ab58b30asoftware/application. In this figure we are showing expression level of Prolactin and Oxytocin with another hormones. Green positive symbol basically shows a positive regulation towards hormone and Red negative symbol show inhibition or negative regulation of hormone. Oxytocin hormone release from posterior pituitary by the stimulating Hypothalamus gland and Prolactin release from anterior pituitary and then interfere with lactose producing cells to produce milk, oxytocin helps in ejection of mother milk, it is contracted with the myoepithelium cell of mammary grand for milk ejection from the alveolar ducts. During the pregnancy Dopamine, serum progesterone and LH hormone level will be decreases for increasing level of Prolactin and Estrogen stimulates the development of mammary gland by the addition of adipose tissue in the breast. PRLR receptors of Prolactin binds to Progesterone NR3C3 receptors for inhibiting the secretion of Progesterone hormone if Progesterone hormone release continuously then lactation level or milk production level will be decreases.

Expression of Prolactin with lowering Progesterone

Prolactin is a well-known hormone which is secreted by the anterior pituitary gland of the hypothalamus and the structure of prolactin is like Placental Lactogen Hormone (PLH) structure and Growth Hormone structure[16,17]. Prolactin has been playing many different roles throughout

the body, during the pregnancy or before few days of pregnancy Prolactin increase their secretion in the body to enlarge breast for breastfeeding to produce milk and colostrum in previous feeding day of the delivery. Colostrum is essential for the baby to protect their immunity basically it is consisting of antibody IgA, IgM and IgG and Prolactin is essential for maintaining homeostasis process in the blood. During the nursing Prolactin and Oxytocin release in a normal range for to stimulate breast alveoli myoepithelium cells, these cells help in milk ejection through the mammary ducts which are connected to breast nipple where the child sucking or feeding mother milk. Women's at time of several months of breastfeeding do not get their periods or not become pregnant during the sexual intercourse due to higher level of Prolactin, higher level of Prolactin suppress the ovarian follicles cycle or also inhibit pituitary hormones mainly LH whenever LH below down as compare Prolactin or other hormones then it is not able to coverup reproductive tract of female due to inhibiting ovarian stimulators that means excess amount of prolactin can be cause amenorrhea (absence of Menstrual cycle) conditions. at time of breastfeeding. Excess amount of Prolactin may cause infertility in the women's due to stopping ovulating/ovulation processes if these like process happens then menstrual cycle will not be stop or may cause irregular periods[18,19]. High level ofProlactin hormone may cause ovulation/ ovulated regularly, but the main facts during higher level of Prolactin the Progesterone hormone will not release in enough amount due to this condition embryo will not be able to implant in the inner lining of uterine wall of the female reproductive system[8].

Inhibiting Prolactin in presence of Dopamine (D-2 receptors)

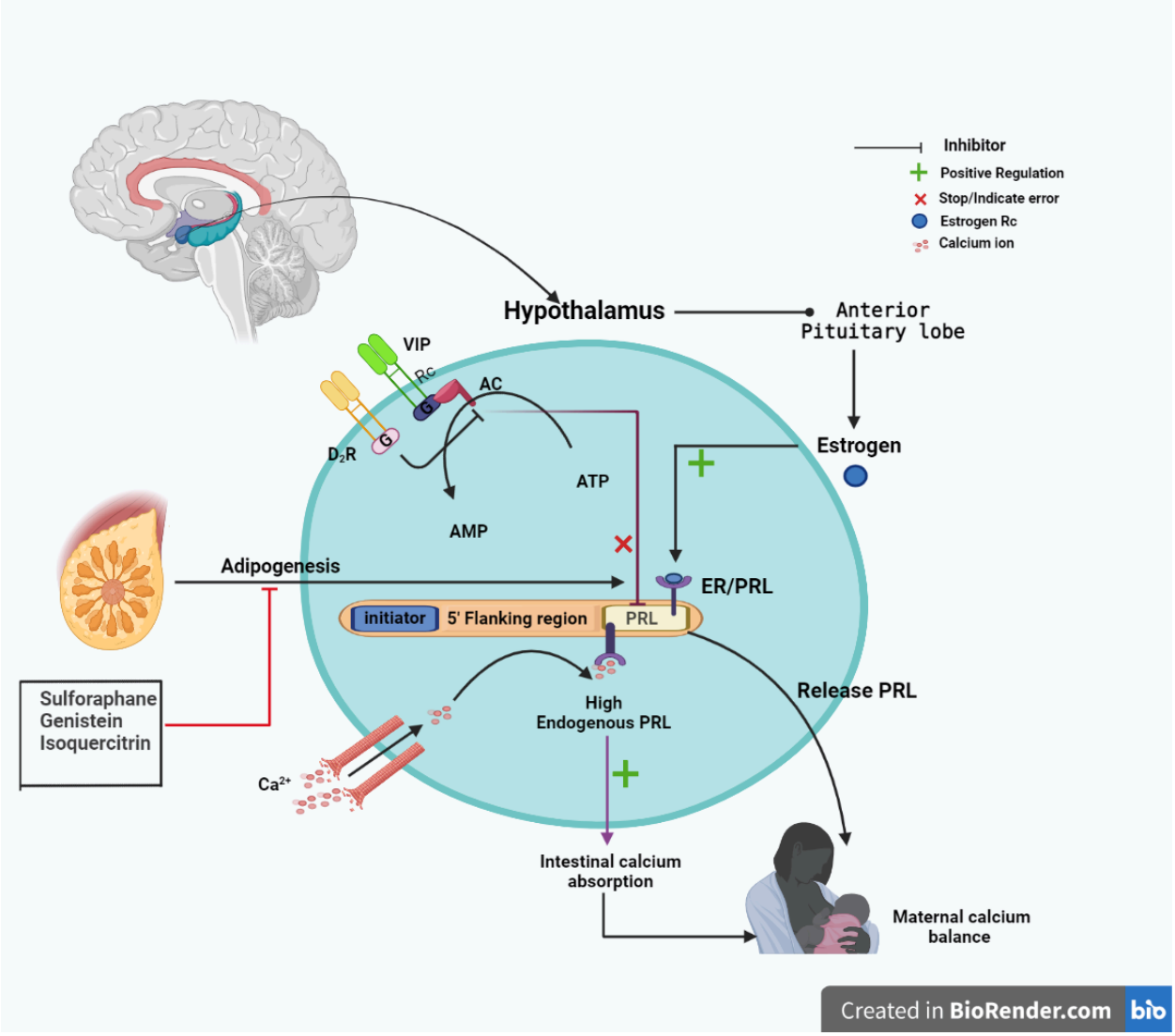


Fig.2: Diagramatically representation basically showing structure mechanism of Prolactin expression in the presence of Dopamine (D_2R) which is react as a negative regulator. Estrogen and thyrotropin are the positive regulator which are helps in regulation of prolactin secretion. Adipogenesis is a pathway process of formation or differentiatation of new adipocytes cells from preadipocytes mature cells. Adipocytes are the mjor components for breast feeding milk as like more adipoctyes cell secrete milk in higher amount.Sulforaphane/Genistein and other inhibitors compounds that are used for controlling the pathway of adipogenesis during the lactation period. Dopamine receptor acts on the pituitary cells to inhibits the stimulatory effects of vasoactive intestinal peptide (VIP) on the Prolactin gene transcription and in other side Estrogen hormone relase from anterior pituitary gland for to bounds to the PRL receptor. Then intracellular Ca^{2+} ions binds to Prolactin in the lactotrophs. Calcium ions also helps in signalling regulatory pathway effects of dopamine and VIP mRNA level of Prolactin and releasing of Prolactin. We

can meausre or determine the expression level of prolactin mRNA in presence of calcium ions by the radioimmunoassay that are release during breast feeding of maternal breast.

LH and FSH are the primary hormones which are essential for the regulation of the menstrual cycle of the women. Dopamine and Progesterone hormone are negatively regulating the synthesis process of Prolactin production in the anterior pituitary gland[12,20]. In male Prolactin inhibits the secretion of GnRH that's affects the spermatozoa (mature sperm) or decreased the process of the spermatogenesis it may be cause infertility in the male. The production of the Prolactin is regulated on the gene transcription level that means factors are uses to stimulate the production, always downregulate gene transcription factors inhibits the production of prolactin but upregulate gene transcription factors initiate the process of Prolactin synthesis[21].

We know that dopamine binds to the D2 receptors on the cell surface of prolactin producing cells in the anterior pituitary gland to inhibit the secretion of prolactin hormone. This happens due to the fact that dopamine D2 receptors are G-protein coupled receptors of the sub type "g Alpha i". G-protein coupled receptors are transmembrane proteinaceous receptors present on the cell surface with seven helical subunits meandering across the phospholipid bilayer. Apportion of transmembrane receptor is outside of the cell membrane where extracellular signalling molecules like dopamine bind to the active site which brings a conformational change in the receptor. Gpcr has its carboxylic and in the cytosol of the cell where it is coupled with g protein which is a heterotrimeric protein of 3 subunits: alpha beta and gamma. The conformational change brought by the binding of dopamine in the receptor makes the heterotrimeric g protein dissociate into monomeric alpha subunit and dimeric beta gamma subunit. Once the alpha subunit dissociates, it releases the GDP molecule attached to it into the cytosol and it binds to a GTP molecule as its energy currency to carry out it's its signal transduction[6]. This activated and associated alpha subunit now binds to a cell surface enzyme called adenelyl cyclase to inhibit its enzymatic activity. An active adenelyl cyclase converts ATP into cyclic AMP which is required to activate a protein kinase enzyme responsible for activating that transcription factor responsible for the transcription of PRL gene to produce prolactin. The inhibition of adenylyl cyclase by the alpha subunit of g protein inhabits the signal transduction which ultimately leads to the transcription of PRL gene into prolactin hormone[6,22]. Therefore, the binding of dopamine-to-dopamine D2

receptor inhabits the cellular biosynthesis of prolactin and ultimately in habits the secretion of prolactin hormone by the anterior pituitary gland.

Dopamine consists D-2 receptors and Dopamine basically secreted in continuous manner from the dopaminergic neuron cells which are located in the arcuate nucleus of Hypothalamus in the anterior pituitary which are visible through infundibulum region, these types of pathways can be called tuberoinfundibular pathway passes. When Dopamine released from the pituitary gland at the terminal downside of the nerves, it's receptor (D-2 receptors) react or interact with the lactotrophic cells of the Prolactin by the intracellular signaling and inhibits the secretion of prolactin hormone, Dopamine is a main source for suppressing the prolactin synthesis in the absence of pregnancy. Estrogen does not affect the secretion of Prolactin as like that progesterone inhibits the level of Prolactin by the stimulating dopamine in the hypothalamus, it's behave as positive regulator for Dopamine[12,23].

The emerging role of prolactin in female – selective pain

Recent studies have revealed that the mechanism through which pain persists is very different among males and females which applies to both animals as well as humans. Studies related to humans and animals show the direct relation of the degree of pain with the menstrual cycle, menopause, etc. Prolactin is seen to entail female-specific regulation of the transient receptor potential (TRP) and also the regulation of other channels in sensory neurons is observed especially the ligand-gated channels[8]. Potentially the prolactin and its receptor the Prlr are involved in this because PRL is present in different cell types which depend on the sex, different phases of the menstrual cycle, in the gestation or pregnancy phase as well as in lactation period. A Study done on a female mouse showed sensitization in sensory neurons when got subjected to exogenous and fully processed non-modified human PRL. PRL is also seen to generate hyperalgesia in female or male mice. Mechanical hypersensitivity was observed only in female mice when injected with PRL injection[7]. Estropus phase has no role to play in the PRL sensitivity. The upregulation of PRL is seen in a sex-dependent fashion when incision surgery and inflammation is seen, especially inside the spinal cord. Emerging data reveals that the

prolactin (PRL) signaling at its cognate prolactin receptor (PRLR) in primary afferents promotes nociceptor sensitization and pain in a female-selective fashion.

Cabergoline

Cabergoline is a commercial drug that mimics the effects of the neurotransmitter dopamine by acting as an antagonist to D_2 dopamine receptors[12,24].

Cabergoline is used to prevent the excessive release of prolactin hormone.

We know that dopamine is also known as the happy hormone which is a neuro-stimulatory or amount as well as a neurotransmitter which helps in the communication of neurones by acting as a chemical intermediate in the synapses. Since dopamine in habits the release of prolactin hormone from anterior pituitary gland, drugs like cabergoline can be used to emulate the inhibition of lactogenic hormone prolactin which is responsible for production of milk in women during pregnancy and spermatogenesis in men. This inhibition is done to treat excessive blood prolactin levels that leads to problems like galactorrhea in women which is characterized by spontaneous flow of milk from the breasts[7,12,25].

Pharmacodynamics

The D2 receptors that are present on the cell surface of lactotroph cells of the pituitary gland are affected by this drug which prevents them from secreting prolactin. Other than D2 receptors cabergoline also binds to seratonin receptors like 5HT 1a, 5HT 2a, 5HT 2b and 5HT 2c receptors. This drug however has an antagonistic effect on the seratonin receptor 5HT 7 and the adrenergic receptors: alpha1 and alpha2[20].

SCIENTIFIC LITERATURE: DOPAMINE AND ITS RECEPTORS

We know that dopamine binds to the dopamine D2 receptors present on the surface of prolactin secreting cells of the anterior pituitary gland. The binding of dopamine inhibits the secretion of prolactin by these cells. Since dopamine receptors are GPCRs, they use g-proteins to carry out the signal transduction to inhibit the transcription of the PRL gene[26], to prevent prolactin

production. Drugs like cabergoline mimic dopamine and act as agonists to the dopamine D2 receptor to inhibit prolactin secretion to treat hyperprolactinemia[7,10].

OBJECTIVE

In this study, we aim to analyze the affinity of cabergoline to the dopamine D2 receptor by comparing the virtual docking score of cabergoline to the D2 receptor with the virtual docking score of the natural ligand dopamine to the same receptor. We aim to further test cabergoline drug's specificity to D2 receptor, by comparing the docking score of cabergoline-D2 receptor complex and cabergoline-D1 receptor complex. In addition to the docking scores, we can further compare the various complexes by studying the intermolecular interactions of the ligands with the binding sites of the receptors[8,26].

PROCEDURE

- Download PDB files of 3D protein structures of Dopamine receptors D1 and D2 having the aforementioned PDB IDs from the protein databank
- Open D2 receptor and D1 receptor protein files on PyMOl and remove extra atoms like water molecules and bound ligands. Save the file as D2.pdb and D1.pdb respectively
- Download the 3D molecular structures of dopamine, and the drug cabergoline with the aforementioned SDF IDs from Pubchem.
- Open PyRX, and upload the D2.pdb and set it as "macromolecule". Upload the dopamine file using "Open Bable" feature and minimize its virtual energy. Set it as "ligand".
- Use the Autodock Vina feature to proceed for a virtual molecular dock. Use the 3D grid to encapsulate the macromolecule, such that all its residues are covered.

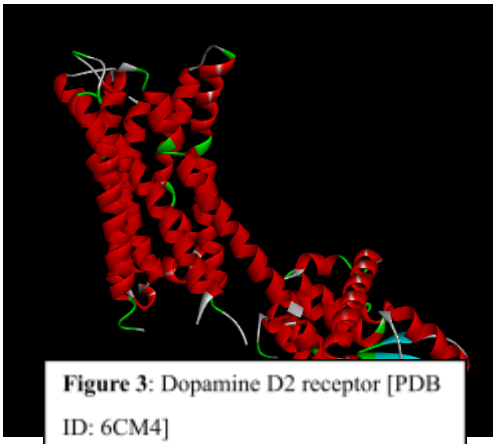


Figure 3: Dopamine D2 receptor [PDB ID: 6CM4]

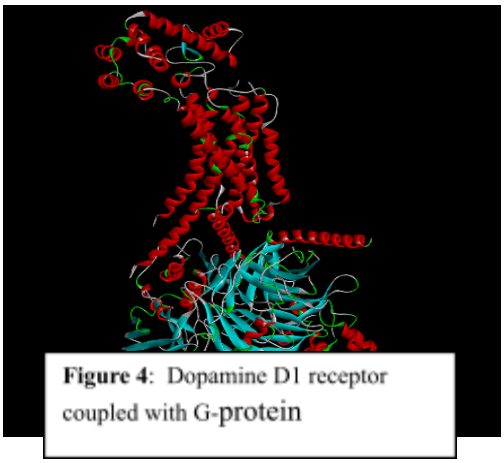


Figure 4: Dopamine D1 receptor coupled with G-protein

- Run the docking between the ligand and the macromolecule.
- Note the docking score. The top-score with the orientation 0 is the highest possible docking score, as this is the orientation at which the ligand has the highest affinity for the macromolecule.
- Upload the file for cabergoline drug and set it as ligand. Keep D2 receptor as the macromolecule.
- Run the docking between the ligand and the macromolecule.
- Note the docking score.
- Upload D1.pdb and set it as the new macromolecule.
- Run docking between dopamine and D1 receptor, and between cabergoline D1 receptor.
- Note the Docking scores
- Download the PDBQT files of each molecule
- Open Discovery studio and upload PDBQT files of dopamine with D2, cabergoline with D2, Dopamine with D1, and cabergoline with D1.

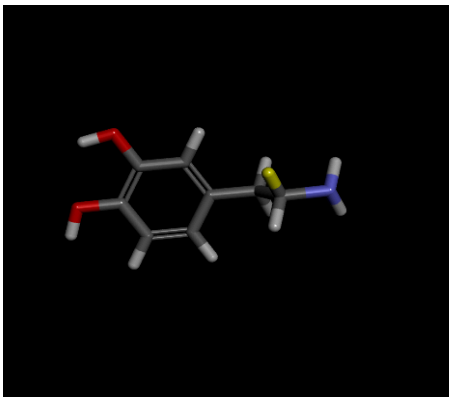


Fig: 5. Crystal structure of Dopamine

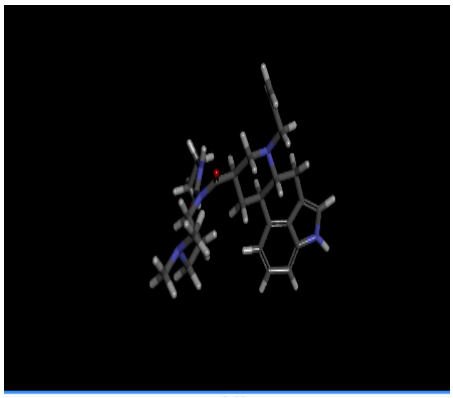


Fig:6. Crystal structure of Cabergoline

OBSERVATIONS

Discovery studio allows the user to visualize the intermolecular interactions of a chosen ligand with the binding site of a macromolecule. One can even find the catalytic amino acids of the bonding site of the receptor by selecting the residue directly bonded to the ligand. By displaying the amino acid sequence, the user can pin-point the residues' location and even edit it accordingly. The user can see the hydrophobic interactions, H-bonding and other non-covalent bonding between the ligand and the macromolecule[3,4].

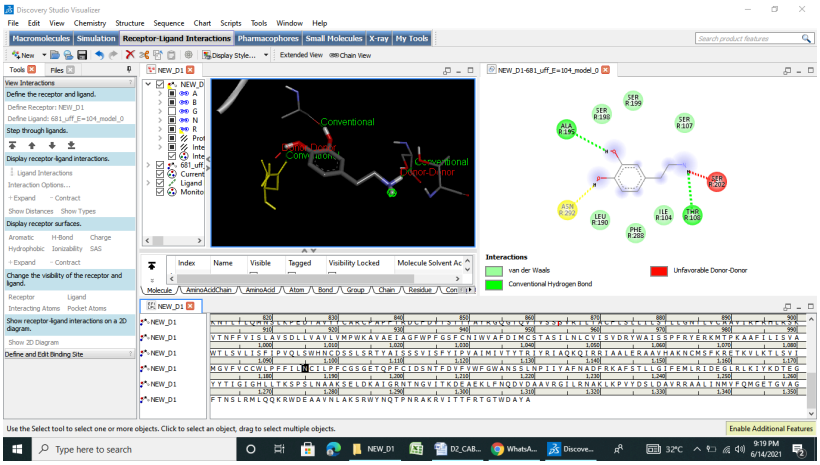
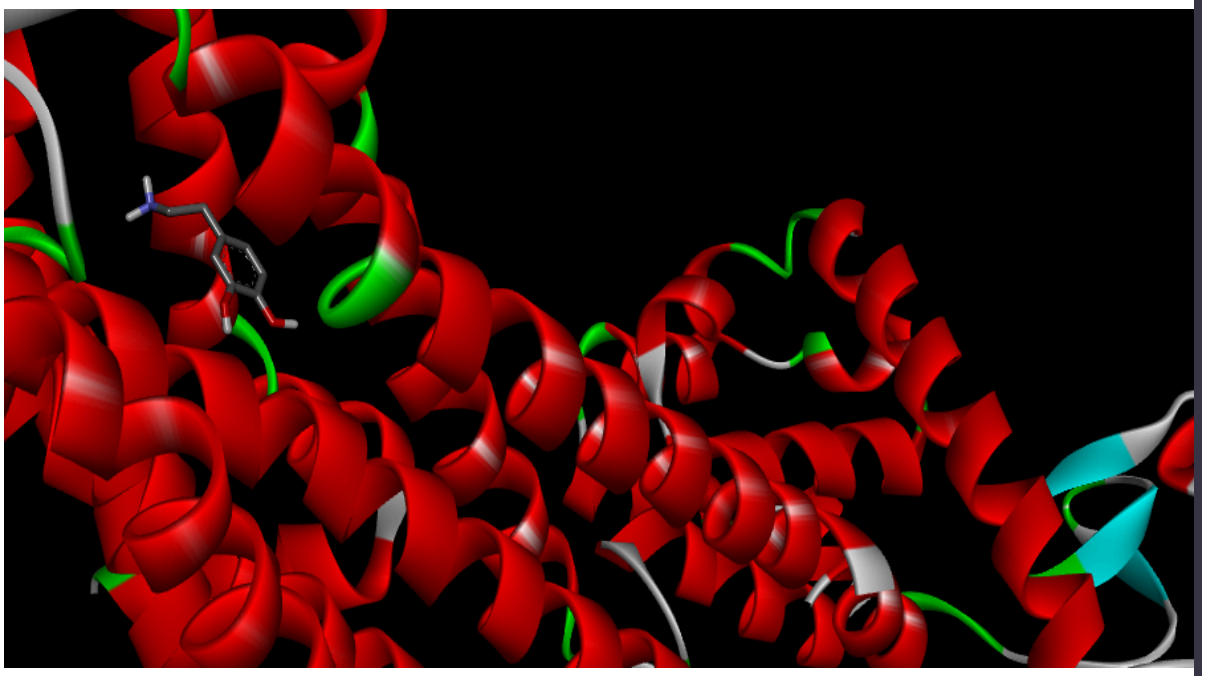


Fig:7. Here we are showing docking of the receptor-ligand interaction in presence of Hydrophobic, Van der waals, Conventional hydrogen bonds and Unfavorable donor-donor bonding by using Discovery Studio visualizer docking software[Free Download: BIOVIA Discovery Studio Visualizer - Dassault Systèmes (3ds.com)](). This is a 2D visualizer software which used mostly in pharmacological department

for to check aromatic, non-aromatic ring, stability of bonds between the ligand and macromolecules and also, we can change the visibility of the tagged molecules those are lightly visible in interaction site.

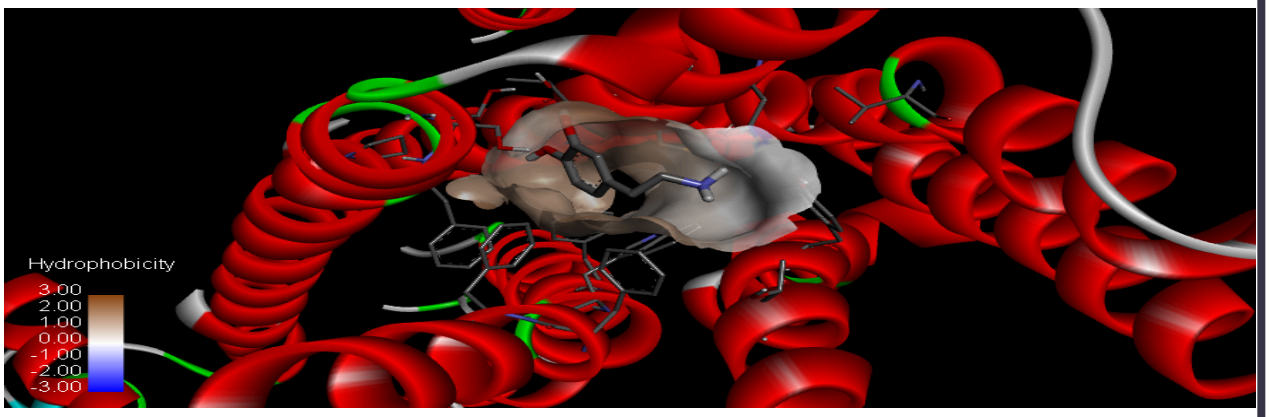
The first complex we encounter is the dopamine-D2 complex. We observe that the ligand dopamine has bound to region between the 7 alpha helices, which tells us the location of the binding site of the D2 receptor.

Fig:8. receptor-ligand complex



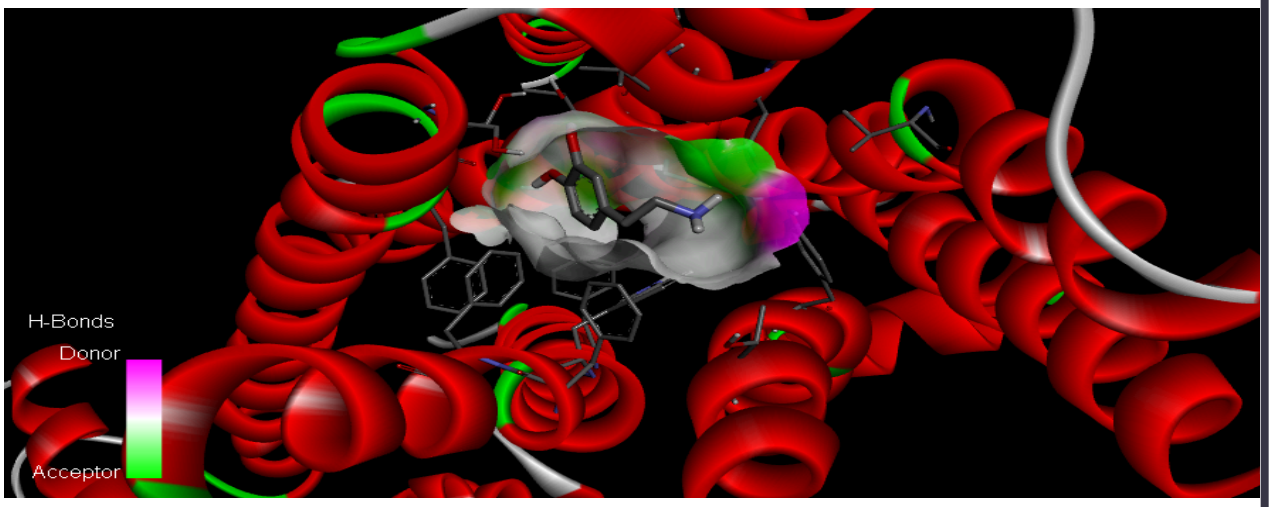
We can see the interactions of the ligand dopamine with the D2 receptor, and note that the binding site is hydrophobic, which means any potential drug candidate that has to mimic the natural ligand dopamine must be a non-polar molecule.

Fig:9. hydrophobic nature of the binding site (dopamine)



We can study the hydrogen bonding between dopamine and the binding site. The pink cloud represents donor residues, while the green cloud represents the acceptor residues. We can use this information to choose a drug candidate that donates hydrogen to the green acceptor residues and accepts hydrogen from the pink donor residues.

Fig:10. H-bonding interactions of hormone- receptor complex



We can also view the specific amino acids interacting with dopamine in 2D. Dark green represents hydrogen bonding, while light green represents Van der Waal's interactions . Pink bonds mean Pi-alkyl bonding.

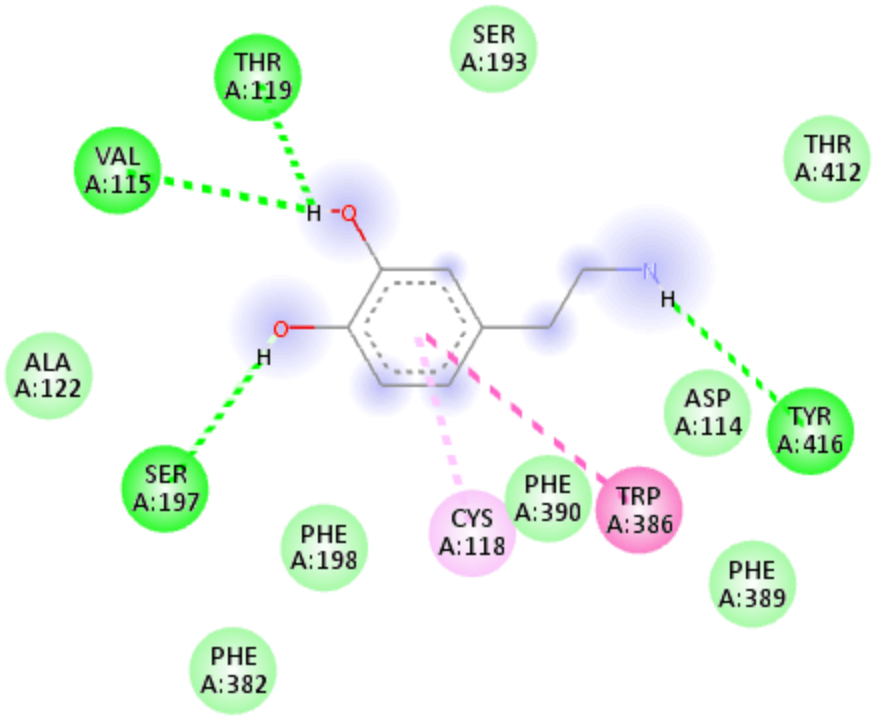


Fig:11. Hormone-receptor interactions at the binding site

We can compare the dopamine's n-binding to D2 receptor with cabergoline and D2. We observe that cabergoline also binds in between the 7 alpha helices that form the binding site of the D2 receptor.

The docking score of cabergoline is higher than dopamine which means it binds more efficiently with the receptor than the natural ligand.

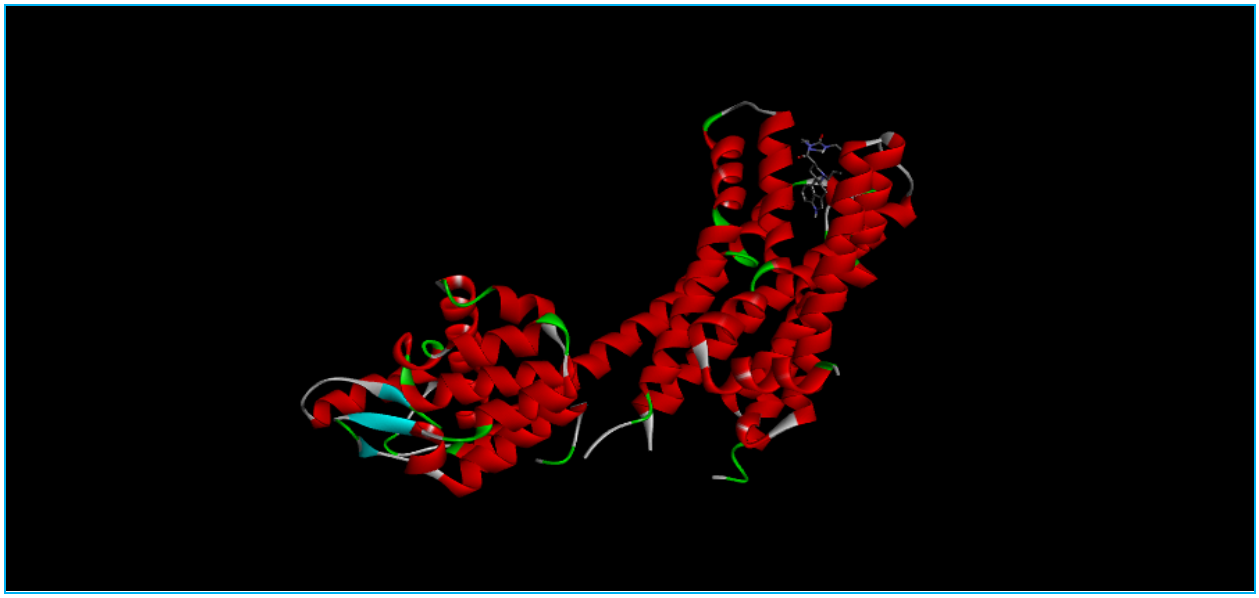


Fig:12. Receptor-drug complex crystal structure

Just like Dopamine, cabergoline also is a non-polar molecule with more than one aromatic ring that fits in the hydrophobic surrounding of the receptor.

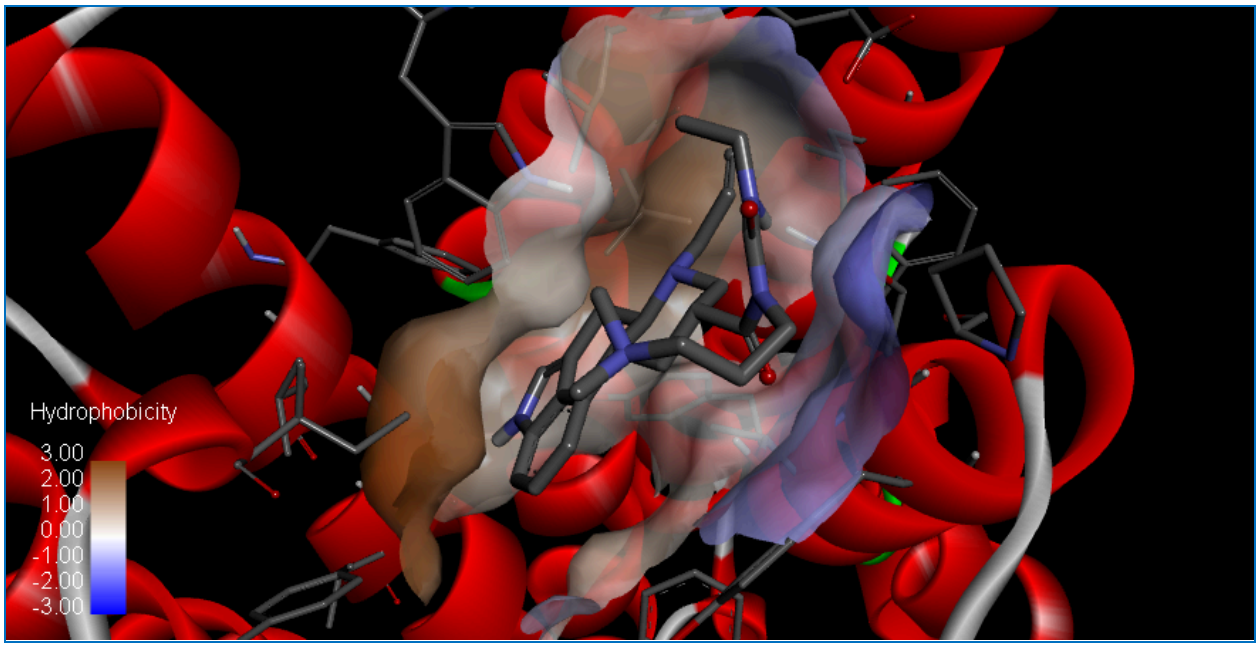


Fig:13. Hydrophobic nature of the drug binding site (cabergoline)

Cabergoline also indulges in multiple hydrogen bond accepting and donations with the amino acids with donor groups shown in pink, and amino acids with acceptor groups.

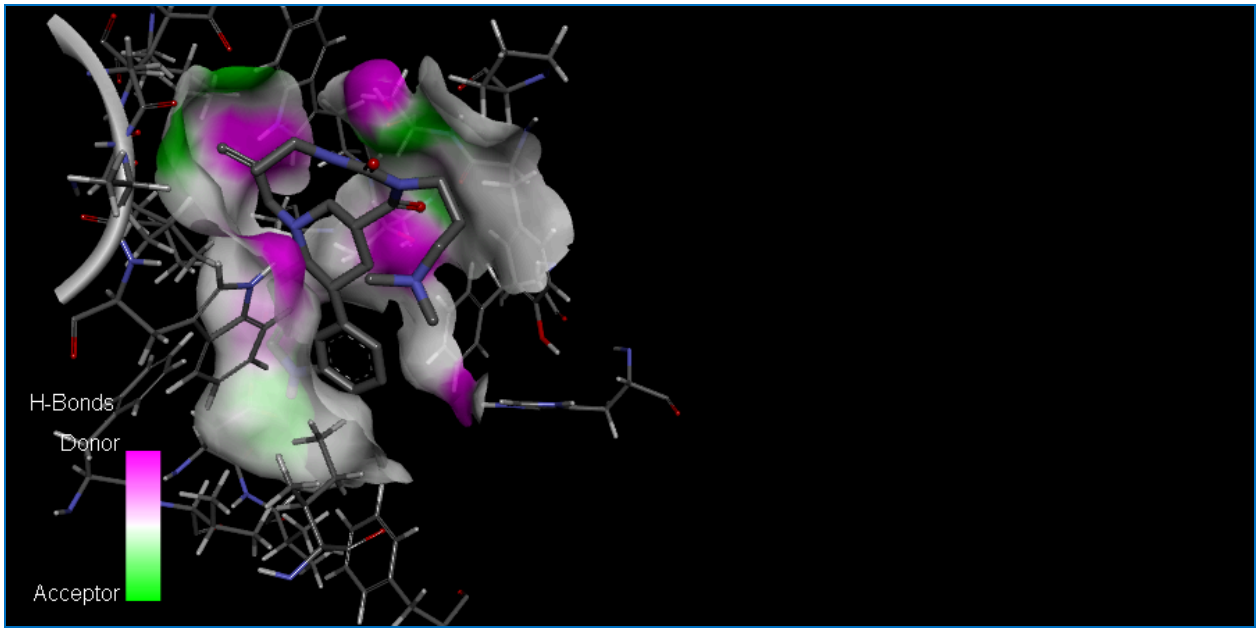


Fig:14. H-bonding of drug with binding site of the receptor

The 2D diagram shows the interactions and their types with specific amino acids of the receptor.

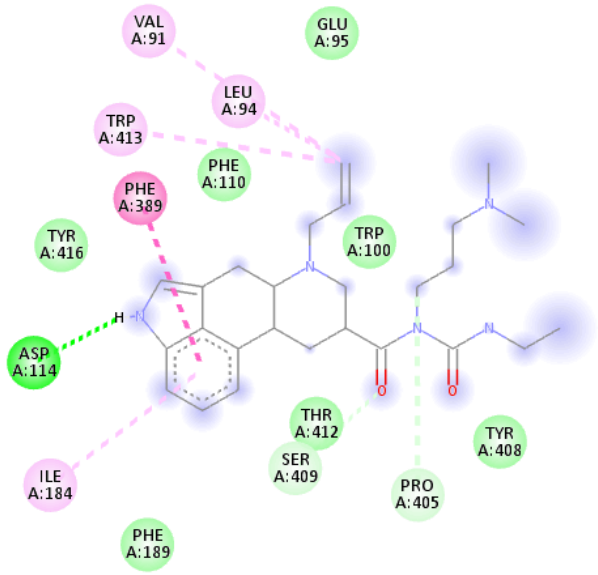


Fig:15. drug receptor interactions at binding site

Furthermore, to study the affinity of cabergoline to D1 and D2, we first study the binding of dopamine to D1 receptor and se that it has almost the same binding score as the dopanie-D2 receptor complex, confirming that dopamine is specific to both.

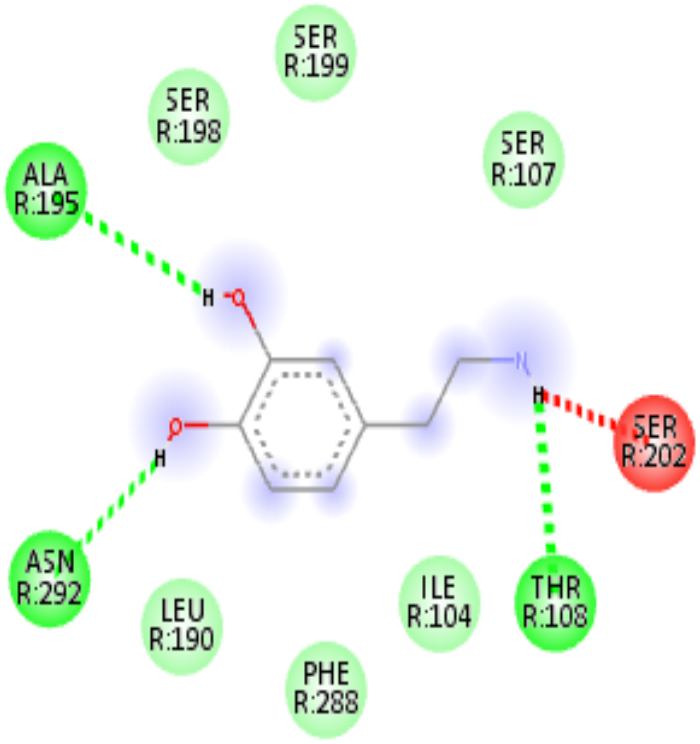


Fig:16. Receptor binding model with other active amine groups.

We then observe the complex of cabergoline to D1 and realize that it has a lesser docking score than the cabergoline-D2 complex, confirming that it has a higher affinity to D2 receptor.

Also, on 3D visualization, we observe that cabergoline doesn't bind to the active binding region of the D1 complex. This means it has a low affinity to this receptor.

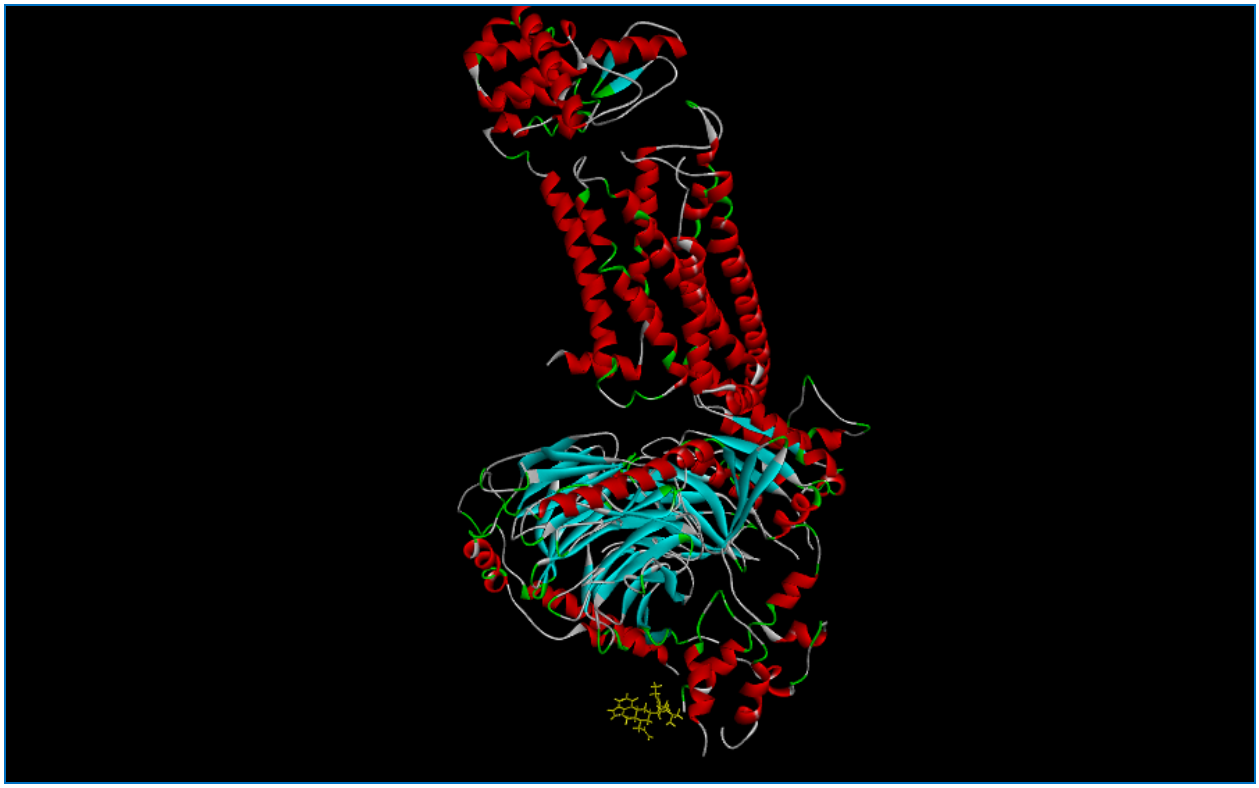


Fig:17. failed binding of cabergoline to the D1 receptor - active site

Table: In this we are showing receptors of Dopamine and Cabergoline drug and its docking score/binding site[8].

Ligand-receptor complex	**Maximum Docking score (negative)**	**Does the Ligand bind to the binding site? (yes/no)**
Dopamine-D2	6.6	Yes
Cabergoline-D2	8.4	Yes
Dopamine-D1	6.2	Yes
Cabergoline-D1	7.5	no

Results

The observations are at par with the published literature in the theory that cabergoline has better affinity to the D2 receptor than dopamine. The observations also confirm that cabergoline has a higher affinity to D2 receptor than D1 receptor[20,24].

DATABASE USED

- **PDB (Protein Data-Bank)**: PDB files for 3D protein structures of dopamine D1 receptor complexed with ligands and G-protein [7JOZ.pdb] and dopamine D2 receptor complexed with ligand [6CM4.pdb].
- **Pubchem**: SDF files for 3D ligand structures of natural neurotransmitter dopamine [Conformer3D_CID_681.sdf]and drug cabergoline [Conformer3D_CID_54746.sdf].

SOFTWARE USED

- **PyMOL (Python Molecule):** molecular visualization and editing software
- **PyRx (Autodock Vina + Open Babel):** molecular docking and virtual screening software
- **Discovery Studio**: molecular visualization and computational Biology software
- **BioRender:** Used for making Scientific depict diagram

FUTURE PROSPECTS OF PROLACTIN

A couple of recent studies support the idea that prolactin might be a foremost contributor to breast cancer advancement and could be an aim for new drugs in counter to numerous forms of the disease. A well-known researcher from VCU Massey Cancer Center Dr. Charles Clevenger, MD, Ph.D., and his laboratory brought to light a new, altered configuration of the prolactin receptor which is called the human prolactin receptor intermediate isoform (hPRLrI) that seems to promptly drive breast cancer. It is observed by the researchers that this modified form of the prolactin receptor interacted with additional forms of the receptor to turn benign breast cells into malignant ones, and the existence of hPRLrI in breast cancer cells remained allied with triple-negative breast cancer, a quick rate of cell reproduction, and unfortunate outcomes[8,26].

The immunohistochemical breast cancer tissue microarray data along with RNA sequencing analyses using “The Cancer Genome Atlas” (TCGA) recognized that higher hPRLrI expression acquaintances with triple-negative breast cancer. These studies suggest that hPRLrI, which in combination with hPRLrI is involved in breast cancer transformation, and represents a novel

oncogenic mechanism, " they say. The researchers note that, despite the fact that the role of prolactin in the development of the mammary gland is known, there is still a discussion about its role in the development of malignant. For example, although epidemiological studies show that high levels of prolactin are associated with an increased risk of breast cancer, patients with hyperprolactinemia are at a higher risk of the disease. It was stated by Clevenger that it was noticed that the prolactin is entangled in the production of the milk and shows the characters of the presence of hormone that may diagnose breast cancer, associate director of Advanced Oncology at Massey and chair of the Department of Pathology at the VCU School of Medicine. "By understanding how the prolactin receptor that connects breast cancer with a new therapeutic strategy and simplified agents can be developed to effectively treat the disease," Clevenger said, these findings also prove that future drug development strategies targeting hPRLrI will eventually speak to advanced disease diagnostic programs for breast cancer as well. Separate research published in the month of May issue of Endocrinology, the group of Clevenger's inhibited tumor growing in preclinical representations of ER-positive breast cancer by means of an HDAC6 inhibitor, a drug that blocks a protein in the context of prolactin[15,21,22,27].

In this study, the researchers found that the prolactin-regulating function of Stat5 is dependent on the state of the enzyme histone deacetylase-6 (HDAC6) and the gene of HMGN2. Their work showed that the estrogen receptor (ER) was high and almost fully interacted with Stat5 and was locally involved in HDAC6 and HMGN2. This suggests that estrogen receptor and prolactin receptor interactions do occur via Stat5 activation with the onset of breast cancer. Treatment of ER-positive breast cancer cells with an HDAC6 inhibitor dramatically slowed tumor development. Clevenger said that "A global analysis of gene expression, once again showing that prolactin is strongly associated with breast cancer growth, may be facilitated by treatment with an HDAC6 inhibitor,". Clevenger has conducted research on whether prolactin can affect the growth and development of breast tumors. In further research, he plans even more trials and development of breast cancer drugs with prolactin, as a primary target.

BIOTECHNOLOGY CHALLENGES

1. Designing and effectively receiving out the necessary prolactin products in the laboratory.
2. Pilot-scale production and analysis of statistical surveys.
3. Success in producing and fabricating bio-edited prolactin.
4. Problems or challenges that arise while moving to new markets.
5. Design must compete with the growing pace of new products and services emerging[9,21].

PROLACTIN PRODUCTS WITH LOW RISK AND HIGH SENSITIVITY FACTORS

It has been observed that in dogs and primate’s prolactin plays an important part in luteotrophic complex. Increased levels of prolactin in human beings hinders steroidogenesis and luteinization of granulose cells. More information of vulnerability of luteal on the prolactin is known in the receptors of prolactin knockouts which shows deficiency in normal function of the luteal and

therefore are sterile caused because of low rate of ovulation, Oogenesis aberrant and the failure of implantation[8,28].

During pregnancy Prolactin is vital for luteal cell hypertrophy and progesterone biosynthesis. In addition to this luteal function, prolactin-R provides many functions for granulosa cells, as well as oocytes. In addition to its luteotrophic role, there is evidence found in the rat that prolactin can be a luteolytic, and also induce programmed cell death in corpora lutea. Prolactin's luteolytic effect, which is apparently mediated by CD3-positive lymphocytes, that increases the expression of the membrane form of the Fas ligand, which is known to mediate luteal cell death via the Fas receptor. In the rat, three generations of corpora lutea can be observed on the ovaries. It is proved that prolactin can act in a cleansing operation by using a structural regression model, the oldest of them. At the time when prolactin exerts this effect then it should duly noted and highlighted that the corpora lutea are non-functional[6,26].

The mechanism by which prolactin can act as both luteotrophic and luteolytic is still unclear. It is assumed that at this critical moment between two periods of prolactin exposure during the estrous cycle, rat luteal bodies acquire the ability to express monocytic chemoattractant protein-1, after interaction with prolactin in the proestrus, inducing luteal cell death.The findings suggest that prolactin has an influence on reproductive behavior. A person with high prolactin levels is associated with psychosomatic reactions, including pseudopregnancy. There is Prolactin-R, which is the ventromedial core of the hypothalamus, in the area where female sexual behavior is monitored[17,28].

Concurrently, it has been that there is an increase in electrical performance of local neurons present in the iontophoresis of the prolactin. Whereas in the rats, the accurate action of the prolactin has been staggered through the multiplicity of the designs of the experiment. Such as when it's provided through the rats third side of ventricle of progesterone-primed ovariectomized estrogen, prolactin lordosis prevalence is declined, an index of receptivity of the sex. Where in, when it is provided through the ratsmidbrain of estradiol-treated ovariectomized, sexual receptivity is intensified by the prolactin. There is a requirement of prolactin in the proliferation of mitogen restoring of the lymphocytes. There is a requirement of prolactin in the proliferation of mitogen restoring of the lymphocytes. The cells of the Nb2 which is procured from the undeveloped T lymphocytes, are mitogenic dependent performance of the prolactin. Although this feature is set out as the basis for a increased sensitivity and determined bioassay for the prolactin[4,17]. Nevertheless, there is no agreement on the part of prolactin in hematopoiesis.

Conclusion

Prolactin is a short peptide hormone which is essential for human to balance their hormonic system and reproductive organ such as, breast, Uterus or Uterine wall, Cervix for implantation of embryo and level up other hormone. Prolactin hormone act as luteotrphic hormone because of it is maintain corpus luteum functions after six days of human mating. HPL may cause irregular periods and infertility in women it may impact on the quality of life. Cabergoline is a commercial

drug that mimics the effects of the neurotransmitter dopamine by acting as an antagonist to D_2 dopamine receptors. This drug used for controlling excessive amount of Prolactin. Observations also confirm that cabergoline has a higher affinity to D2 receptor than D1 receptor.

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