Potential Opioid Addiction Therapeutics

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Abstract

Throughout the last thirty years, a severe opioid epidemic has arisen due to the excessive consumption and abuse of these addictive narcotics. Opioids are currently the best analgesic known to man, however the effects of opioids are not all beneficial; they are extremely addictive and are deadly when taken in high doses. Since opioids began rising in popularity in the 1990's as a prescribed pain-reliever, opioid deaths have skyrocketed. These circumstances have caused the need for the development of both a potent, non-addictive pain reliever and also a way to treat patients with an opioid addiction. To solve this problem, we used computational methods and structural analysis to investigate the μ-opioid receptor binding cavity and its unique interactions with four different ligands: morphine, heroin, fentanyl, and naloxone. From the results, we have created a criterion of interactions that a potential opioid therapeutic should have.

Introduction

Chemically, opioids have been defined as anything that binds to the opioid receptor. Medically, their purpose is to provide relief for those who are struggling with chronic pain. There are a wide range of opioids that have become increasingly available to the public recently. From the illegal drug heroin to the synthetic fentanyl to the legally prescribed oxycodone, hydrocodone, codeine, and morphine, opioids are very widespread. Similar to most other drugs, opioids can either be classified as natural or synthetic. Natural opioids, such as codeine and morphine, are derived directly from the poppy plant. On the other hand, synthetic fentanyl is manufactured in a laboratory setting. Methods of use have also been developing over time. While opioids were once simply injected or consumed in a tablet or capsule, individuals now have access to delivery methods such as patches, liquids, lozenges, pain pumps, and nasal sprays. The issue within these various methods of opioid use is that individuals do not know how to properly administer their drug of choice. Consuming an excess of anything usually does not lead to positive consequences. In the case of opioids, an overabundant dosage will lead to a severe overdose possibly causing death. Some of the non-deadly side effects include sleepiness, constipation, loss of consciousness, nausea, shallow breathing, and slowed heart rate. As the opioid epidemic took off the in the 1990s due to the prescribing behavior of physicians (Barnett 2017), people began using opioids less for pain relief and more for the recurring euphoric feeling to satisfy addictive cravings.

Pain Management

When it comes to treating pain, especially chronic pain, there is no universal method or medication that is guaranteed to

produce complete relief. Other than opioid use, individuals may partake in alternative forms of pain management such as over-the-counter medications, physical therapy sessions, acupuncture, surgery, or injections. Understanding pain from a biochemical standpoint is a complex concept not catered to the general public. Opioids work by binding to proteins called opioid receptors located on neurons throughout the body. When this occurs, opioids inhibit pain signals sent from the body through the spinal cord to the brain. The "holy grail" of opioid research is to find an agonist that relieves pain without the euphoric effects that cause addiction. Addiction is commonly understood as a brain disease in which recurrent exposure to a substance alters its structure and function, ultimately contributing compulsive drug-seeking behaviors (Lyden 2019). Opioid addiction has had a detrimental effect on members of our society, making the need for a solution ever-pressing.

History and Socioeconomic Impact of Opioids

While opiates have been used as analgesics since the 1700s, prescription opioids were not developed for medical use until rather recently in the 1900's. They were first used sparingly for extreme pain, but became more common in medical offices with the release and FDA approval of oxycodone in 1995 (Lyden 2019) to treat the "epidemic of untreated pain" (Alexander 2015). The marketing of opioids made them seem relatively harmless, as they did not fully or accurately address the addictive qualities of the drugs (Alexander 2015). In addition, doctors often misprescribed the drug, as clear outlines of application and dosing of the drug were not determined (Alexander 2015). Under the belief that opioids were safe, prescriptions skyrocketed, and as a result, so did rates of addiction and overdose. The US saw a 300% increase in opioid prescription from 1991-2009 (Alexander 2015) during the "first wave" of overdose deaths, in this case due to prescription opioids (CDC 2018). Following this, people started to overdose on heroin, which is often used by addicts if they are not able to access prescriptions (CDC 2018). In 2013, the "third wave" of overdose deaths began, this time involving synthetic opioids like fentanyl (CDC 2018). In 2016 alone, over 214 million prescriptions were written and 64,000 people died due to opioid overdose. Overdose deaths have been increasing for all opioid types, but the greatest increase in overdose deaths has been seen in synthetic opioids, such as fentanyl (CDC 2018). In 2017, about 15 of 100,000 people died due to overdosing (CDC 2018), with 60% of these deaths being caused by synthetics (CDC 2018).

All opioid users, especially those who have become addicts, are susceptible to the risk factors associated with such lethal drugs. The opioid crisis has hit impoverished areas especially hard, as those living in poverty face a higher risk of addiction than those who are not (NIDA 2017). History of substance abuse, depression, use of psychotropic medication to treat mental illness, and being of young age can all increase likelihood of misuse (Lyden 2019). Those prescribed opioids also face a greater risk of addiction, with middle aged and elderly patients suffering from chronic pain most likely to be

medically exposed (Chou 2015). As of recent, hospitalization rates due to opioid overdose have increased more for women than men: 75% as compared to 55% (Lyden 2019).

Pros and Cons of Opioid Use

Aside from addiction, opioids can have other adverse physical effects. According to a report completed by Roger Chou, the medication has the potential to increase likelihood of bone fracture, heart attacks and erectile dysfunction. However, at this time, opioids are the best form of pain relief if their side effects are not considered. There are many people suffering from acute and chronic pain daily, and choose to risk the consequences of opioids in hopes of relief. Pain due to illness, injury, operation, etc., not only causes physical suffering to the patient, but emotional, mental and economic suffering as well (Sessle 2011). A 2008 Canadian survey revealed that those suffering from pain felt a reduced quality of life and relationships, problems in the workplace, and increased rates of depression and suicide (Sessle 2011). In the U.S., annual healthcare costs due to pain exceed $100 billion, including loss of compensation due to pain (Sessle 2011). These costs are likely to go up, as the population of middle aged and elderly Americans, who are most likely to suffer from chronic pain, is increasing (Sessle 2011). The use of opioids can diminish economical, physical and emotional side effects of pain, and because they are the most powerful pain reliever as of yet, would be effective at doing so if side effects like addiction, bone fracture, and heart attacks could be avoided.

Opioid Receptor Characterization

There are three types of opioid receptors: μ, δ, and κ. Studies have shown that when knockout mice are genetically modified to not express the μ-receptor and given morphine, they do not experience any analgesic or addictive effects (Loh 1998). Because of this, the μ-opioid receptor (u-OR) is thought to be responsible for the pain-relieving effects of opioids and has become the primary pharmacological target of the opioid receptors. The μ-OR is a G-protein coupled receptor, or GPCR, which is a transmembrane receptor that relays signals from the extracellular matrix by coupling with a G-protein. G-proteins have three subunits: alpha, beta, and gamma. The G-proteins create secondary messengers, which can elucidate a variety of effects on the body based on the type of receptor and signal. The receptor can respond to different ligands, with each ligand inducing a range of effects, and with the optimal ligand having the maximal effect. The principal endogenous ligand for the μ-opioid receptor is B-endorphin, which has the highest binding affinity to the protein out of the endogenous opioids. The natural effect of endorphins leads to a feeling of euphoria coupled with lessened anxiety and a reduced feeling of pain. These molecules help with pain management, activate the reward system, and modulate euphoria (Sprouse-Blum 2010). Other endogenous opioids are endomorphins, which have a high affinity to the receptor, enkephalins, and dynorphins which have a low affinity (Holden 2005). There are also many agonists that bind to the receptor including DAMGO, fentanyl, methadone,

morphine, hydrocodone, and codeine. Antagonists include naloxone (narcan), and diprenorphine. These agonists and antagonists can also interact with other opioid receptors - resulting in the side effects seen when taking opioids such as increased euphoria, decreased consciousness, constipation and respiratory suppression.

Structural Analysis

The μ-opioid receptor has the characteristic 7 transmembrane helices found in GPCRs, with three intracellular loops and three extracellular loops. The binding cavity of the protein is more exposed compared to other GPCRs, leading to a higher accessibility by ligands. This potentially causes more rapid effects and faster ligand dissociation. This could be a reason why opiate overdoses are very quickly reversible by administering naloxone. In the crystal structure developed by Manglik et al., there are fourteen residues within four angstroms of the bound ligand (B-FNA). Eleven of these residues are conserved through all opioid receptors, and 3 are specific to the μ-receptor - Glu229, Lys303 and Trp318, meaning that these residues could be responsible for the differences in ligand affinity and physical effects of the different types of opioid receptors. The most important residue may be His297. In a mutagenesis study, when replaced with Ala, there was a complete loss of opioid binding. It is believed to form a hydrogen bonding network with water and the ligand phenyl hydroxyl group (Manglik 2012). Conversely, mutation of Asn150 resulted in an increase in binding of ligands, meaning this residue could be a way that the protein mediates ligand release and prevents the binding affinity from being too high. Asp147 is also important; it's negative charge on the R-group is thought to interact with the nitrogen group on opioids. Other key residues include Tyr326, which when mutated to Phe resulted in lowered ligand affinity.

Mechanism of Action

The mechanism of action of opioids is very complex, activating and inhibiting a variety of molecular pathways. The result of these molecular actions is the characteristic analgesic and addictive effects of opiates. The primary way in which the μ-opioid receptor functions is by activating potassium ion channels and negatively inhibiting calcium ion channels (Al-Hasani 2011). When the potassium ion channels open, K^+ is allowed to flow out of the neural cells, hyperpolarizing them. The result of this is that action potentials are less likely to fire, limiting neuronal excitability. Calcium ions work in the brain to release neurotransmitters from their synaptic vesicles into the synaptic cleft where they can bind to receptors to cause an effect. When calcium ion channels are inhibited, this results in a deficiency in neurotransmitter signalling. These two effects in combination result in less activity in the brain, ultimately resulting in a reduction of nociceptive transmission.

The signalling pathway is as follows: The opioid binds to receptor, and the alpha subunit of the G-protein exchanges GDP for GTP. The alpha-GTP subunit complex then dissociates from the other two G-protein subunits, and interacts with other proteins. The alpha subunit inhibits adenylyl cyclase, resulting in less cyclic AMP production (Pathan 2012). Cyclic AMP is a secondary

signalling molecule that is involved in many pathways, most commonly known as an allosteric activator of protein kinase A, an enzyme that phosphorylates other proteins involved in metabolic processes.

Opioids have the ability to relieve intense pain and provide physical, economical and emotional relief to those experiencing discomfort. At the same time, the drug can be highly addictive, and in many cases, lead to overdose and death. Our initial research on opioids has lead us to wonder if there may be a way to separate the addictive nature of the drug from its pain relieving abilities, and what a ligand with such qualities may look like. To determine this, we docked four known binders in order to investigate bond length distances, molecular composition, residues and types and number of interactions present with structures that bind successfully. Our results provide an outline for potential opioid therapeutics.

Methods

Docking of Compounds

We began our research by selecting small molecules known to bind with the μ-opioid receptor. We chose to investigate the binding characteristics of four ligands: morphine, heroin, fentanyl and naloxone. Morphine, heroin and fentanyl are all opioid agonists whereas naloxone is an opioid antagonist.

Docking was performed with the μ-opioid receptor PDB code 5C1M (Huang 2015). Ligand files were made using the DrugBank database (Wishart 2018) SMILES format, then using the SMILES to PDB converter (NCI) developed by the NIH to create 3D ligand PDB files. AutoDock Tools (Morris et al 2009) was used to create ligand files with the correct partial atomic charges and formulate the docking grid box. The grid box was created around all of the key residues for opioid binding. The dimensions were set to 20 points in the x, y, and z directions with a spacing of 1.0 Å, for a total of 9,261 grid points. The box was centered at 0, 16.278, -60.889 for all ligands. AutoDock Vina (Morris et al 2009) was used to dock the ligands into the opioid binding cavity. PyMOL (Schrödinger) was used for visualization, analysis, and manipulations of the protein and docking conformations. The poses with the lowest RMSD scores for each ligand were selected for docking and further analysis of interactions with the key amino acids located in the μ-opioid receptor binding cavity.

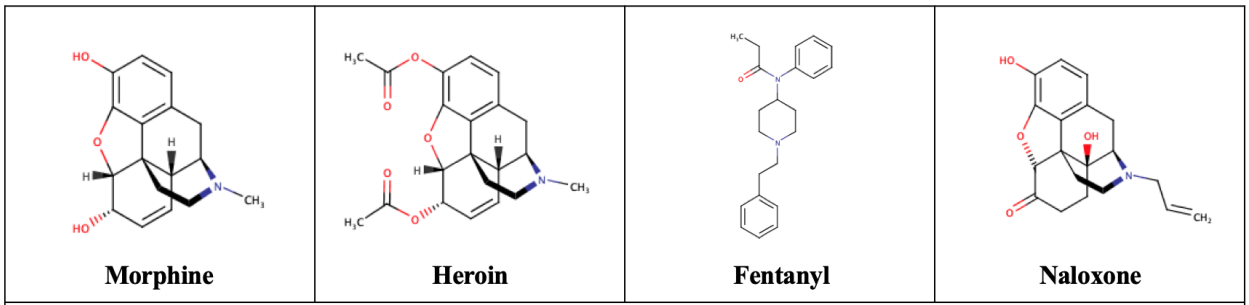

Fig 1. Chemical structures of the four ligands investigated in this project: morphine, heroin, Fentanyl, and naloxone. Representations were created using MarvinSketch and Microsoft Office PowerPoint.

Docking Analysis

After docking, bond length distances were created using the Measurement Wizard tool in PyMOL. The molecular composition of the ligand pharmacophores and the residues which it interacts with gave us insight into the types of interactions these ligands are engaging in. For example, if a ligand phenyl group was within 3.9 Å of a non-polar amino acid, we considered this a hydrophobic interaction. Conversely, if a ligand amine group was within 3.3 Å from a residue carboxyl group, we considered this to be a hydrogen bond. These interaction thresholds were decided by using values from literature of other similar docking studies (Lewis 2011). The key residues for opioid binding analyzed were Asp147, His297, Tyr326, and Asn150. We also were interested in the three residues specific to the μ-OR: Glu229, Lys303, and Trp318 (Mansour 1997) to determine if the difference in physiological response between the opioid receptor isoforms could be explained by these variant amino acids. However, the docking analysis was not limited to only interactions with these specific residues, other amino acids within the distance thresholds were also considered.

Results

In morphine, heroin, and naloxone, the ligand amine group is positioned towards Asp147 and Tyr326, while the ligand oxygens are oriented towards the entrance of the binding cavity. The methyl groups of heroin orient downward and away from Tyr147 and Tyr326. Phenyl groups on either end of fentanyl create interactions: the top phenyl group interacts with Val143 (not pictured) and the bottom phenyl group with H297. The naloxone hydroxyl group, bonded with D147 and Y326, points upward, as does its carbon chain. Ligand oxygens on all ligands except fentanyl point to the top of the protein toward the extracellular region of the protein and entrance to the binding cavity.

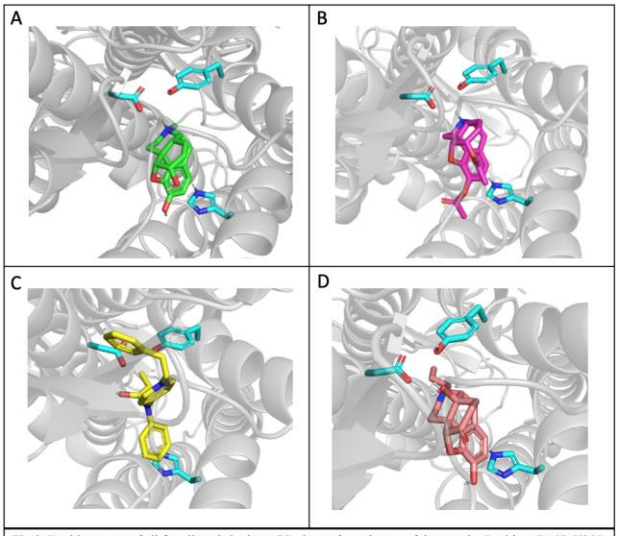

Fig 2. Docking poses of all four ligands in the μ-OR shown from the top of the protein. Residues D147, H297, and Y326 are shown as sticks in blue, each in the same location in all representations. **A.** Morphine shown with green carbons. **B.** Heroin shown with pink carbons **C.** Fentanyl shown with yellow carbons. **D.** Naloxone shown with salmon carbons.

Hydrogen bonding is present between all ligands docked here and the mu opioid receptor. Hydrophobic interactions are present when fentanyl and naloxone are docked in the protein. Naloxone has the greatest number of bonds, with the smallest average distance (3.08 Å) between ligand and residues in these bonds. Morphine has the next smallest average bond distance at 3.2Å. Fentanyl and heroin both have average bond distances of 3.3Å, the largest value. There are six interactions seen in the docking of heroin, and three seen with fentanyl and morphine.

Morphine

When morphine is docked into the mu opioid receptor active site (Fig 3a), we observe hydrogen bonding interactions between both Asp147 and Tyr326 and the

morphine amine group. This observation is supported by literature; the negative charge on the R-group is expected to interact with the nitrogen group on opioids (Manglik 2012). We also see another hydrogen bond form between Tyr148 and a ligand ring oxygen.

Heroin

Similar to morphine, we see heroin engage in hydrogen bonding between its amine group and Asp147 and Tyr326, as well as between Tyr148 and a ligand oxygen (Fig 3b). As seen in Figure 1, the only structural differences between morphine and heroin are the two acetyl groups present on heroin where there are hydroxyls on morphine. The methyl groups on the end of these acetyl groups have interactions with the μ-OR active site not seen with morphine. One group is within 4 Å of both Trp318 and Val300, and the other methyl is within 4 Å of Val236.

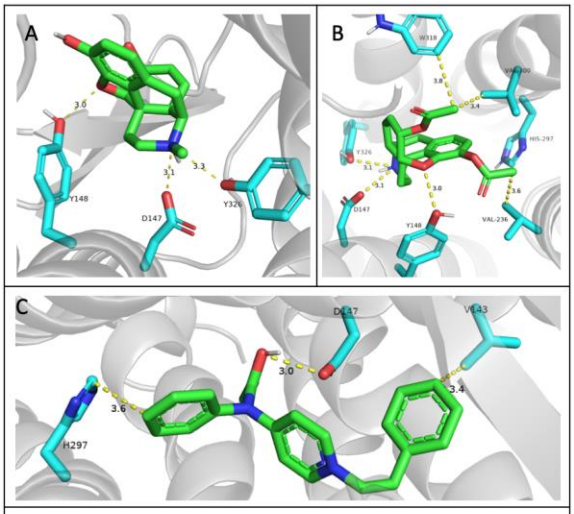

Fig 3. Ligands (**A.** morphine, **B.** heroin, **C.** fentanyl) docked in the active site of the mu-opioid receptor with distance measurements shown. Ligand carbons are shown in green, amino acid carbons are shown in blue, and the protein is shown as a cartoon in grey. The representation was created using PyMOL.

Fentanyl

Fentanyl has the most unique structure of the four ligands; it is planar structure and has two exposed hydrophobic phenyl groups. When docked, we observed a hydrogen bond form between Asp147 and a ligand oxygen. We also see two hydrophobic interactions - one phenyl group interacts with a His297 ring

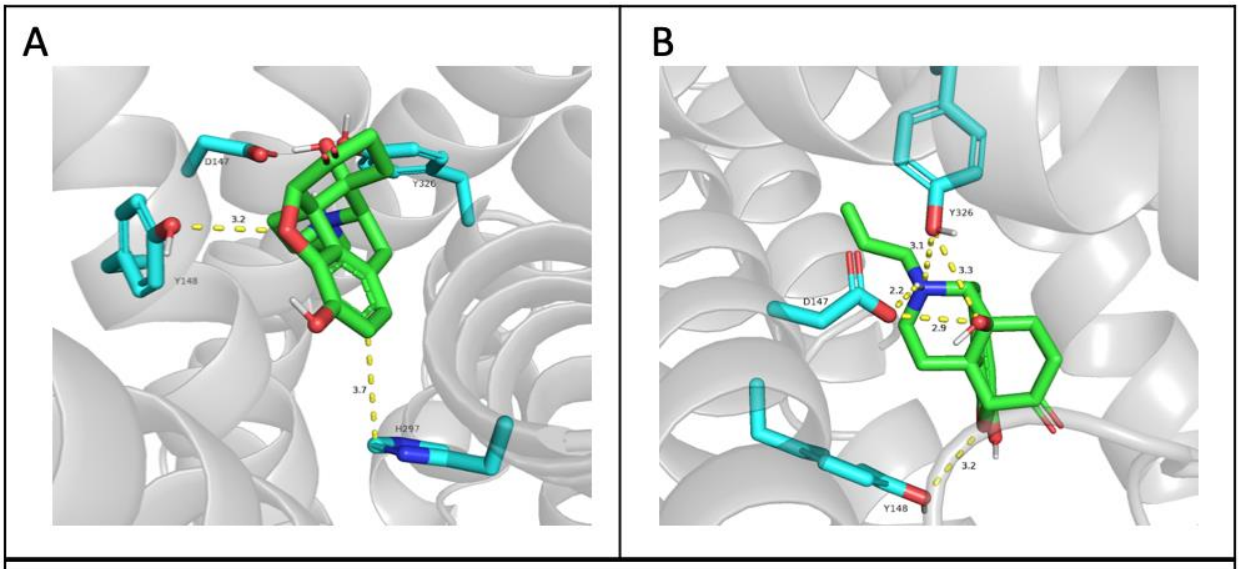

Fig 4. Naloxone docked in the active site of the mu-opioid receptor with distance measurements shown. The two pictures are the same pose taken from different angles. Naloxone carbons are shown in green, amino acid carbons are shown in blue, and the protein is shown as a cartoon in grey. The representation was created using PyMOL.

carbon, and the other phenyl group interacts with Val143.

Naloxone

Naloxone has similar structural elements to morphine and heroin, such as the four six-membered rings. It also differs with an elongated carbon chain from the ring nitrogen, a ketone functional group where morphine has a hydroxyl and heroin has an acetyl group, and an additional hydroxyl group (Fig 1). Just like heroin and morphine, we observe hydrogen bonding with Tyr148 and an interaction between the ligand amine group and Asp147 and Tyr326. However, we see both Asp147 and Tyr326 also forming hydrogen bonds with a naloxone hydroxyl group not present on either heroin or morphine (Fig 4b). In addition, we also observe a hydrophobic interaction between His297 and a naloxone ring carbon (Fig 4a).

Discussion

In all of the docking results, we observe the ligand engaging in hydrogen bonding with Asp147. This residue is also mentioned in literature as a key residue. In a mutagenesis study, a mutation of Asp147 resulted in a five-fold decrease in binding affinity (Manglik 1997). Because of this, we believe that any potential opioid therapeutic must have a polar interaction with Asp147. From the same mutagenesis study, when His297 was mutated to alanine it resulted in a complete loss of opioid binding. In our dockings, we found this residue to have hydrophobic interactions with fentanyl and naloxone. This could possibly be an explanation as to why fentanyl is more potent than morphine or heroin, and naloxone can preferentially bind to the receptor over other opioids and reverse overdoses. While we didn't see any polar interactions with the ring nitrogen on His297, it may be involved in the formation of a charge network with other residues in the binding cavity or interact with the ligand through a bridge with a water molecule.

The three residues unique to the μ-OR active site are Glu229, Lys303, and Trp318. In all of the docking results, we did not see any significant ligand interactions with any of these residues except for one hydrophobic interaction between W318 and heroin. However, we did observe that all three of these residues are located near the entrance of the binding cavity (Fig 5). Because of this, we hypothesize that these residues could be involved in substrate selectivity and may mediate which ligands are allowed to enter the binding cavity.

Morphine and heroin have the same structure, except heroin has two acetyl groups where morphine has hydroxyls (Fig 1). We hypothesize that the increased potency of heroin could be accounted for because of different interactions in the active site by these two acetyl groups. In the docking

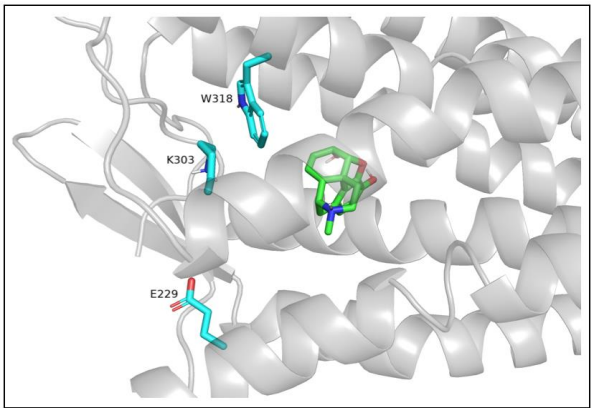

Fig 5. Residues specific to the μ-opioid receptor shown in blue. The ligand shown in green is morphine.

results, we observe one acetyl carbon 3.6 Å away from Val236 and the other acetyl carbon 3.4 Å away from Val300 and 3.8 Å from Trp318. These non-polar interactions not present when morphine is docked could possibly explain the increased potency of heroin compared to morphine. Fentanyl has a very different structure than the other opioids studied in this report. Morphine, heroin, and naloxone all have bridged structures and oxolane groups, whereas fentanyl is planar and has two exposed phenyl groups. We hypothesize that these hydrophobic interactions may be responsible for increased potency of fentanyl since these exposed hydrophobic groups are unique to fentanyl, although more work needs to be done to confirm this hypothesis.

When docked, naloxone had a different binding profile that the other three opioids, which could explain why it is an antagonist and competitive inhibitor that has a greater affinity than other opioids and makes it a fast-acting and effective antidote for overdoses. We saw an electron sharing network formed between Asp147, Tyr326, and an oxygen and a nitrogen of naloxone. This sharing of electrons may confer stability to the ligand:protein complex, making the binding of naloxone more thermodynamically favorable than other ligands. When analyzing potential therapeutics, this type of binding profile could be desirable. More investigation is needed to determine the reason why naloxone does not induce an analgesic effect, but we believe that a naloxone analog could be a launching point for the development of a novel therapeutic.

Conclusion

When reviewing small molecules as potential opioid addiction therapeutics, we are looking for compounds that are strong binders without inducing the typical euphoric effect felt when under the influence of opioids such as morphine and heroin. By comparing ligand structures, physiological effects, and binding profiles, we have constructed a list of criteria for a potential therapeutic. Our first criteria is that a ligand must have a polar interaction with Asp147. In all docking results we see a hydrogen bond form between an amine group on the opioid and the carboxyl group on Asp147. Next we believe that the ligand should have an exposed hydrophobic group, like the phenyl groups on fentanyl or acetyl groups on heroin. Lastly, the therapeutic should form an electron sharing network much like naloxone, possibly making it a stronger binder. While there is much more work to be done in solving the opioid epidemic, we believe that having an understanding of how different opioids interact with the μ-OR active site and how it changes the effects they elicit will contribute to the development of a novel, non-addictive opioid therapeutic.

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