Investigating the Mechanism of Kainate Receptor and Identifying its Inhibitors Using In-Silico Techniques

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The GluK1 receptor, an ionotropic glutamate receptor, operates as a ligand-gated ion channel. Opioids stand as the most potent pain-relieving medications currently prescribed in the medical field. Inhibiting this receptor is anticipated to result in a reduction of dopamine release in the Nucleus Accumbens, effectively preventing the primary factor contributing to addiction. In addition, in the absence of the glutamate, the GluK1 receptor cavity should shrink and expand in the presence of it. We hypothesized that the glutamate binding and unbinding in the receptor cavity should result in major conformational changes (cavity opening and closing activity) in the glutamate binding site. In the current research, we utilized computational techniques, specifically molecular dynamics simulations, to initiate an examination of the GluK1 receptor and its mechanism for binding with glutamate. Additionally, we employed molecular docking methods to predict compounds capable of binding to the glutamate binding site, potentially inhibiting this receptor. Our simulations have highlighted that the primary fluctuations within the receptor predominantly occur during glutamate binding. Lastly, we have also proposed a mechanism for how glutamate binds to the receptor.

1. INTRODUCTION

Numerous neuropathologies originate from dysregulation in the excitatory or inhibitory activities of specific neuronal populations within the brain, including opioid addiction (1). Opioids represent the apex of analgesics currently employed in clinical settings. Their mechanism of action involves binding to opioid receptors distributed throughout the Central Nervous System (CNS), leading to a diminution in the propagation of nociceptive signals (2). The paramount efficacy of opioids as analgesics, however, is attributed not just to their role in pain modulation but also to their capacity to induce dopamine release. Dopamine, a neurotransmitter predominantly secreted by neurons in the Nucleus Accumbens – a brain region integral to the reward system and the reinforcement of rewarding behaviors – elicits sensations of euphoria and well-being (3). Kainate receptors (KARs), belonging to the ionotropic glutamate receptors (iGluRs) subfamily, are essential for both postsynaptic neurotransmission and presynaptic regulation of transmitter release in the vertebrate brain (4). These receptors differ from AMPA and NMDA receptors by producing small-amplitude excitatory postsynaptic currents (EPSCs) with slow kinetics in the hippocampus regions of the central nervous system (5). KARs also play a crucial role in the development of neuronal circuits, interacting with G proteins, and are implicated in various disorders, including autism, epilepsy, schizophrenia, and neuropathic pain. Subunits from the low and high kainate affinity gene families make up KARs (6). While the high-affinity family (GluK4-GluK5) must partner with low-affinity subunits to function, the low-affinity family (GluK1-GluK3) can form both homomeric and heteromeric receptors (7). This assembly produces various receptor configurations, contributing to their diverse functional properties (8).

The functional diversity of KARs is further expanded through RNA editing and alternative splicing (9). In particular, KARs expressing GluK1 are predominantly located in the hippocampus, Purkinje cells, cortical interneurons, and sensory neurons undergo these processes, producing four isoforms of the C-terminal domain and two N-terminal domain isoforms, GluK1-1 and GluK1-2. The GluK1 subunit is only found in the GluK1-1 form, with an extra 15 amino acids in the amino-terminal domain (ATD) (10). This variation may give KARs that carry its unique characteristics. The impact of this N-terminal splice inserts on the modulation of KARs by auxiliary subunits such as Neto proteins remains unexplored.

Gamma-aminobutyric acid (GABA), a neurotransmitter, is secreted by neurons within the Nucleus Accumbens (11). This secretion acts primarily as an inhibitory mechanism, moderating dopamine release (11). Elevated dopamine levels typically induce euphoria; however, GABA's modulatory role prevents excessive dopamine release, thereby regulating this euphoric

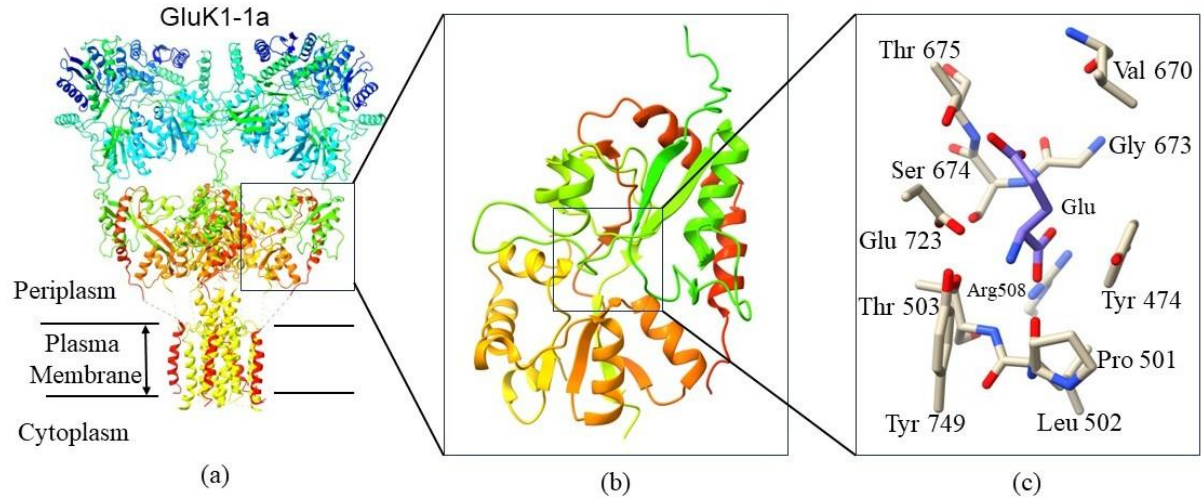

Fig. 1. Different components of GluK1-1a receptor. (a) 3D model of GluK1-1a receptor, (b) glutamate binding domain, and (c) glutamate (purple) bound to the amino acids surrounding it.

response (11). Opioids, known for their analgesic properties, concurrently inhibit the transmission of nociceptive signals and the release of GABA by GABAergic neurons (11). This inhibition allows dopaminergic neurons to release substantial quantities of dopamine, culminating in heightened euphoria (11). This mechanism underlies the profound addictive potential of opioids, rendering countless individuals dependent and unable to cease usage (11). The significant health implications necessitate the development of alternative therapeutic strategies. These strategies could mitigate opioid-induced adverse effects while retaining their analgesic efficacy. Our research focuses on identifying inhibitors of the Kainate (Gluk-1a) Glutamate Receptor, aiming to diminish dopamine production in the Nucleus Accumbens, thus offering a novel approach to address this pressing healthcare challenge.

In the present study, molecular docking and molecular dynamics techniques were employed to investigate the functionality of the Kainate Receptor (GluK1) and to predict potential inhibitors targeting this receptor. We hypothesized that the glutamate binding and unbinding in the receptor cavity should result in major conformational changes (cavity opening and closing activity) in the glutamate binding site. In addition, in the absence of the glutamate, the GluK1 receptor cavity should shrink and expand in the presence of it. Our research unveiled the dynamic behavior of the protein, particularly its interactions with glutamate, in comparison to its interactions with pharmaceutical compounds. The identification of inhibitors for GluK1-1a Kainate receptor and subsequent structural analysis thereof would contribute significantly to advancing our comprehension, thereby facilitating the development of pharmaceutical agents capable of modulating the Kainate receptor and subsequently influencing the associated neural pathways, either propagating or inhibiting them as a consequential outcome.

2. RESULTS

In this study, all-atom unrestrained μs long MD simulations of the GluK1 protein receptor in an aqueous solution were performed to explore the glutamate binding mechanism to the receptor. Experimental studies suggest that protein undergoes significant conformational chances and has a unique glutamate binding mechanism discussed in this section using molecular dynamics simulations. Furthermore, inhibitors against this receptor have also been proposed using molecular docking simulations. The computed root-mean-square deviation (RMSD) values of Cα atoms indicated that the relaxation of these structures requires at least 200ns of simulation time.

Figure 2 shows the cavity formed in the center of the protein. The figure illustrates the difference between the two cavities; one with glutamate is smaller, and the cavity becomes larger in the absence of glutamate. This is because, due to the presence of glutamate, the amino acids surrounding the cavity come close to interacting with glutamate and cause the cavity to shrink. This change in the binding site has also been observed in the GpdQ enzyme.(12,13) The amino acids that form interactions with glutamate are Ala496, Arg667, Asp668, Glu422, Glu719, Ile420, Pro497, Tyr470, Tyr745, and Val666.

According to the MD simulations, conformational flexibility was also observed in the protein. During the 200ns simulation, conformational changes in the loop region of the protein were observed. This shows that the loop opens up in the absence of glutamate to accommodate the glutamate in the cavity. This conformational change is further confirmed in the RMSF graph

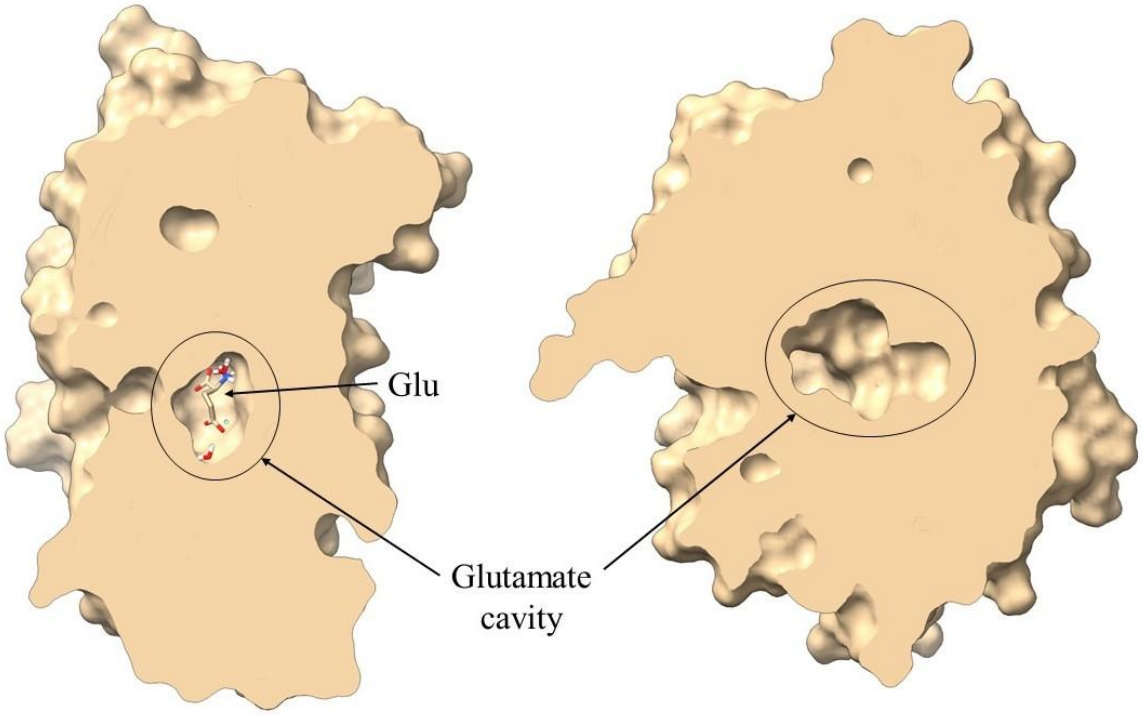

Fig. 2. Section of the surface map of the protein. The X-Ray structure with smaller glutamate cavity is shown in left and the MD simulated structure with larger cavity is shown in right. Cavity constricts in the presence of the glutamate and constricts in the absence of the glutamate.

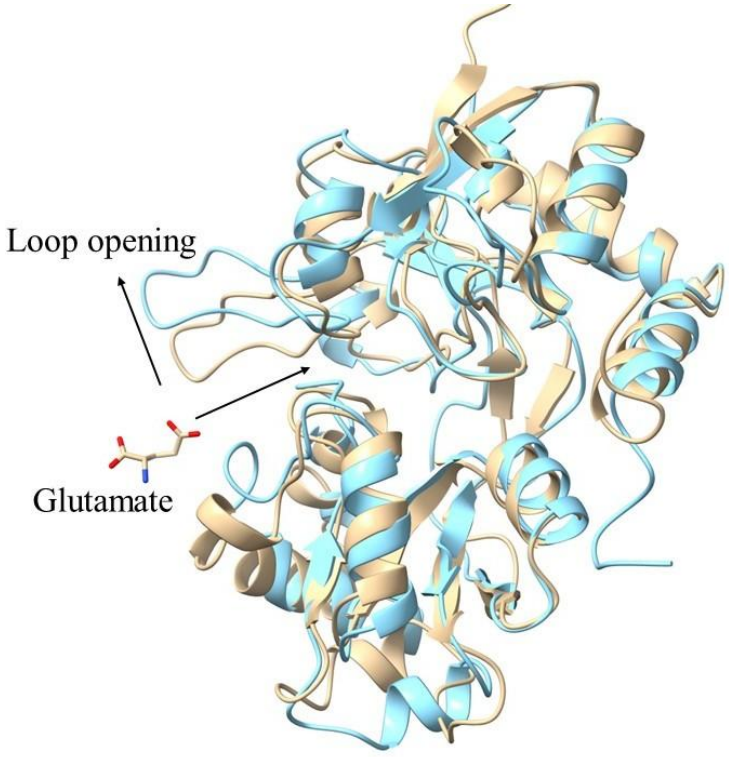

Fig. 3. Superimposed structure of Xray (khaki) and MD simulated structure of GluK1 (cyan). The cyan structure represents the resting (open) state of the protein in which the glutamate is absent and the brown structure represent the active (closed) state of the protein in which the glutamate is present in the cavity.

shown in Figure 4. Based on the RMSF graph, the peaks between residue numbers 580-600 show the conformational changes in the loop opening.

Inhibitors against the receptor: The protein ligand interactions were computed using a protein-ligand interactions profiler (PLIP) webserver, which was used to predict the protein-ligand interactions. Based on this analysis, **Ligand I** forms four hydrophobic interactions with Ile420 (3.61 and 3.64 Å), Tyr470 (3.61 Å), Ala496 (3.87 Å), and Val666 (3.89 Å). In addition, it forms three hydrogen bonds with Tyr470 (2.23 Å), Arg667 (2.81 Å), and Asp668 (3.29 and 3.55 Å). Also, it forms with Tyr470 at a distance of 4.89 Å. **Ligand II** forms four hydrophobic interactions with Ile420 (3.59 and 3.68 Å), Glu422 (3.69 Å), Tyr470 (3.57 and 3.73 Å), Pro497 (3.70 Å), Glu719 (3.77 Å), and Tyr745 (3.92 Å). In addition, it forms three hydrogen bonds with Tyr470 (2.10 Å), Arg667 (3.50 Å), and Asp668 (3.80 Å). **Ligand III** forms four hydrophobic interactions with Ile420 (3.53 Å), Tyr470 (3.60 Å), and Val666 (3.74 Å). In addition, it forms two hydrogen bonds with Arg667 (3.05 and 3.01 Å) and one π-Stacking Tyr470 (4.24 Å). **Ligand IV** forms four hydrophobic interactions with Ile420 (3.53 Å), Glu422 (3.80 Å), Tyr470 (3.56 and 3.97 Å), Pro497 (3.73 Å), Tyr745 (2.37 and 3.95 Å). In addition, it forms one hydrogen bond with Arg667 (3.05 and 3.01 Å) and one π-Stacking Tyr470 (4.87 Å). **Ligand V** forms four hydrophobic interactions with Tyr470 (3.37 Å), Ala496 (3.96 Å), Val666 (3.72 Å), and Tyr470 (2.44 Å). In addition, it forms three hydrogen bonds with Arg667 (3.12 Å) and Tyr470 (4.87 and 5.17 Å) and one π-Stacking Ile420 (3.46 Å).

3. DISCUSSION

Glutamate binding mechanism: The mechanism of glutamate binding in the GluK1 receptor, a type of ionotropic glutamate receptor crucial for excitatory neurotransmission in the brain, is a detailed and intricate process. The proposed mechanism is shown in Figure 5. It begins with the resting (open) state receptor in which the two domains, D1 and D2, are open, Figure 5a. This opening allows water molecules to ingress into the ligand-binding pocket, preparing it for the entrance of glutamate. Once glutamate enters this pocket, it interacts with various interior amino acids through hydrogen bonds and ionic interactions, ensuring a precise and stable binding, Figure 5b. This specificity is vital for correct signal transmission across neurons. Following the secure binding of glutamate, the D1 and D2 domains close around the ligand, encapsulating it and triggering a conformational change in the receptor. This change leads to the receptor's ion channel opening, allowing the flow of ions, typically calcium or sodium, across the cell membrane. This ion flow initiates a cellular response or propagates a neural signal, illustrating the complexity and precision of cellular signaling mechanisms in the nervous system. In the final step, the glutamate is released, and the receptors obtain their original resting (open) state, Figure 5c. Understanding the intricacies of glutamate binding to the GluK1 receptor sheds light on the fundamental mechanisms of neurotransmission and unveils potential therapeutic targets for neurological disorders. As research continues to unravel the complexities of this process, it opens doors to innovative interventions aimed at modulating neurotransmitter activity with precision and efficacy. Thus, delving deeper into the molecular choreography of synaptic communication holds promise for advancing basic neuroscience knowledge and clinical treatments for various neurological conditions.

This study uses molecular docking and molecular dynamics (MD) simulations to find possible inhibitors and explore the binding mechanism of the kainate-type ionotropic glutamate receptor, GluK1. According to the study, the GluK1 receptor experiences substantial conformational changes during glutamate binding and unbinding, particularly in its cavity, which contracts when glutamate is present and expands when it does not. Several ligands that can block the receptor by creating hydrogen-bonding and hydrophobic interactions with important amino acids including Tyr470, Arg667, and Ile420 were found using docking simulations. By lowering dopamine release in the Nucleus Accumbens, these findings give novel inhibitors that could modify glutamate activity and offer therapeutic paths for disorders like opioid addiction. They also shed light on the dynamic nature of the receptor.

4. METHODS

The hexameric 1.9 Å resolution X-ray structure (PDB ID: 8GPR) provided the monomeric version of the E protein structure (10). The Autodock Vina 1.5.6 program was used to investigate the binding positions of ligands to the E protein opening (14). Ten poses, with an exhaustiveness value of 20, were produced for each of the four enzyme-substrate complexes. The hexameric 1.9 Å resolution X-ray structure (PDB ID: 8GPR) provided the monomeric version of the E protein structure (10). The Autodock Vina 1.5.6 program was used to investigate the binding positions of ligands to the E protein opening (14). Ten poses, with an exhaustiveness value of 20, were produced for each of the four enzyme-substrate complexes. The GROMACS-4.5.6 program and the AMBER03 force field were used to do the MD simulations(15). To replicate a physiological ion concentration of 0.154 M, the simulations were carried out in a cubic box filled with TIP3P water molecules. The system was neutralized by substituting specific water molecules with potassium and chloride ions. For 200 ns, the MD simulations were run. The water molecules' bond lengths and angles were constrained using the SETTLE technique, while the remaining bond lengths were constrained using the LINCS algorithm. NPT ensembles with constant values of N, P, and T were used to run the simulations.

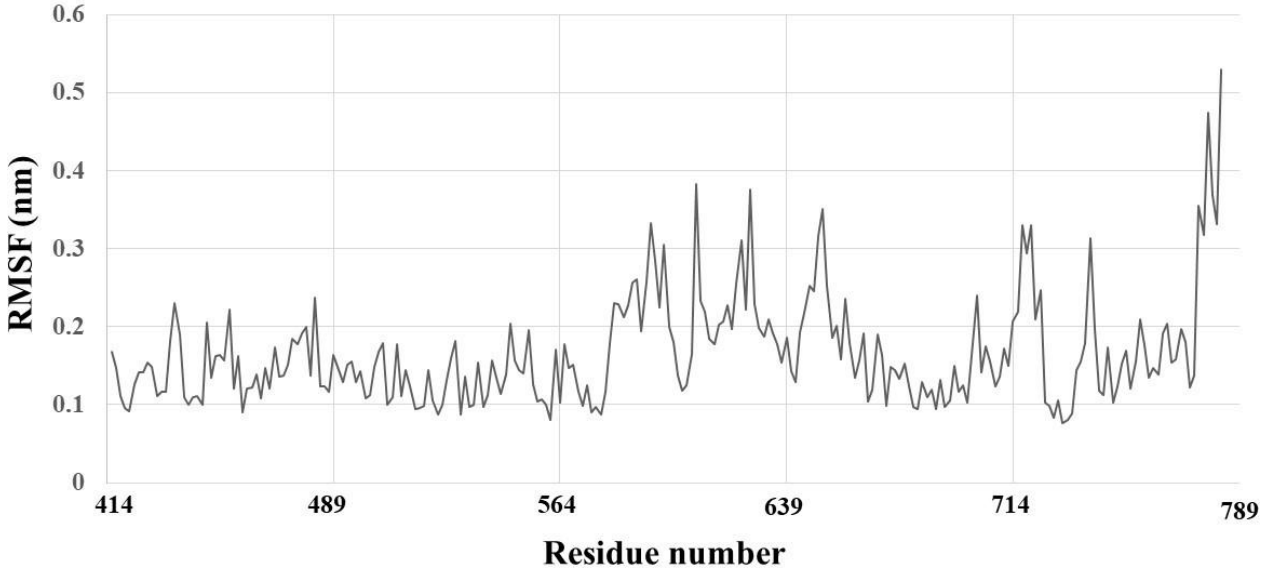

Fig. 4. Root mean square fluctuation (RMSF) in the protein MD simulations. The RMSF shows the fluctuation observed in the protein. The major fluctuations were observed in domain 1 and domain 2, showcasing the cavity opening and closing effect.

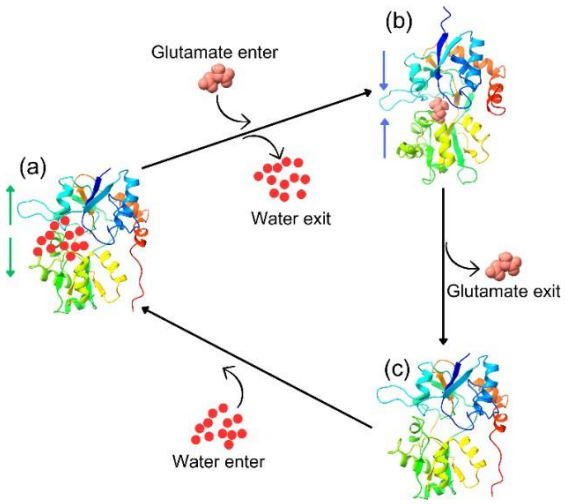

Fig. 5. Glutamate binding mechanism. (a) in the free state the receptor is in free (open) state in which the cavity is filled with water molecules; (b) when glutamate enters the cavity the amino acids (present in cavity interior) interact with the glutamate and results in the shrinkage of the cavity. The cavity shrinkage results in the water release; and (c) the release of glutamate results in the opening of the cavity.

Table 1. Receptor-Ligand Interactions.

Ligand I Interactions	Amino acids	Distances (Å)
Hydrophobic Interactions	Ile420	3.61, 3.64
	Tyr470	3.61
	Ala496	3.87
	Val666	3.89
Hydrogen Bonds	Tyr470	2.23
	Arg667	2.81
	Asp668	3.29, 3.55
π-Stacking	Tyr470	4.89
Ligand II		
Hydrophobic interactions	Ile420	3.59, 3.68
	Glu422	3.69
	Tyr470	3.57, 3.73
	Pro497	3.70
	Glu719	3.77
	Tyr745	3.92
Hydrogen Bonds	Tyr470	2.10
	Arg667	3.50
	Asp668	3.80
Ligand III		
Hydrophobic interactions	Ile420	3.53
	Tyr470	3.60
	Val666	3.74
Hydrogen Bonds	Arg667	3.01, 3.05
π-Stacking	Tyr470	4.24
Ligand IV		
Hydrophobic Interactions	Ile420	3.53
	Glu422	3.80
	Tyr470	3.56, 3.97
	Pro497	3.73
	Tyr745	3.95
Hydrogen Bonds	Tyr745	2.37
	Tyr470	4.87
Ligand V		
Hydrophobic Interactions	Tyr470	3.37
	Ala496	3.96
	Val666	3.72
Hydrogen Bonds	Tyr470	2.44
	Arg667	3.12
	Tyr470	4.87, 5.17
π-Stacking	Ile420	3.46

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