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SODIUM CHANNEL HYDROGEN BONDING IN EPILEPSY: MOLECULAR PHYSIOLOGY AND BIOPHYSICS

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ABSTRACT

The present review provides information about the impact of molecular physiology and biophysics of hydrogen bonding in the activity of sodium channel, and anticonvulsants and therapeutic aspects for epilepsy. Molecular and biophysical mechanisms of epilepsies are required to be identified since epilepsy is a complex neurological disorder or a syndrome and has no effective antiseizure treatment yet for several of the its manifestations/ disorders. Hence, receptor-drug studies with three-dimensional geometry are needed to be designed to understand the role of intramolecular and intermolecular interactions including hydrogen bonding. The hydrogen bonds are quite fundamental for understanding the physiophysics of biomolecules at ultrahigh and true-atomic-resolution. Therefore, the present review is a future direction for conducting further studies using X-ray diffraction patterns and a variety of other molecular physiology and biophysics techniques to unravel the mystery of interactions in epilepsy disorders.

Key-words: Hydrogen bonding, sodium channel, epilepsy, physiophysics, molecular physiology and biophysics

INTRODUCTION

The hydrogen bond has energy varying from 1 to 40 kcal/mol depending upon its environment, geometry and the type of atoms involved (Steiner, 2002), and is though stronger than a van der Waals interaction, it is a week type of bond as compared to other chemical bonds (weaker than ionic bond; and about 1/20 the strength of covalent bond).

The term ' hydrogen bond' was used first in 1912 by T.S. Moore and T.F. Winmill (Moore and Winmill, 1912). Hydrogen bonding is quite a common noncovalent interaction (Głowacki *et al.,* 2013) for slightly positive hydrogen (H+) of a polar covalent bond for making bonding with strongly electronegative atoms (oxygen, nitrogen, fluorine) of a polar covalent bond (a strong dipole-dipole attraction) intramolecularly or intermolecularly. It may occur in organic (e.g., proteins, DNA) and inorganic (associated liquids e.g., water) molecules. It is highly important for making and stabilizing the chemical and biological structures for the manifestation of a variety of functions (Lin *et al.,* 2020).

Voltage-gated sodium channels (Na_V or VGSCs) regulate initiation and propagation of the action potentials in excitable cells (Hodgkin and Huxley, 1952; Hirschberg *et al*., 1995; Yu and Catterall, 2003). The Na_V mutations often cause pathophysiological conditions (Huang *et al.,* 2017) including cardiac arrhythmia (Pei *et al*., 2016), epilepsy (Richards *et al.,* 2018), chronic pain syndromes (Meents *et al.,* 2019), myotonias (Fusco *et al.,* 2015) etc.

The present review provides information about the impact of molecular physiology and biophysics of hydrogen bonding in the activity of voltage-gated sodium channel, and anticonvulsants and therapeutic aspects for epilepsy.

GENERAL BIOPHYSICAL CONCEPTS

It is highly important to understand the basic concepts related to ion channel activities for having idea of the role of hydrogen bonding in ion-channels/ ion channel disorders.

A theoretical study for hydrated sodium ion-phenylalanine clusters Na+(Phe)(H2O)n (n = 0-6; Phe = phenylalanine) relating protein geometries and functionals and ion channels, revealed that the change between quadridentate and pentadentate complexes are from the competition between cation-O bonding and hydrogen bonding (Gao *et al*., 2023).

The related concepts concern to permeability, equilibrium potentials and pumps Nernst equation is quite relevant (Wright, 2004):

Em = RT/zF * log ([ion outside cell]/[ion inside cell])

where Em is the equilibrium potential of the membrane, R is other gas constant (8.314472 $J \cdot K^{-1}$), T: temperature Kelvin, F: Faraday constant (9.65 x 104 C mol^{-1}), Z: for monovalent ion, 2 for divalent and etc., and RT/F in simple form is 61.5 at normal body temperature (Chrysafides *et al*., 2021).

Random movement of ions can be explained by:

Fick's law: $J=-D\,\partial C/\partial x$

(J: molecular flux, D: diffusion coefficient, $\partial C/\partial x$: concentration gradient &

Nernst-Planck equation explains:

$J=-D\,(\nabla C + Fz\,RT\,\nabla\varphi)$.

Where, conservation $\partial C/\partial t + \partial J/\partial x=0$, reaches to

diffusion equation determination:

$(\partial C/\partial t = \partial/\partial x\,(D.\partial C/\partial x)$.

Sodium channel and other ion channel proteins in the membrane serve as conductor molecules, and Gauss's law surface charge (Ɛ: permittivity of free space) as: $\Sigma=E\varepsilon_0$. 'Surface charge density' could be electric charge amount-q in an area-A as: $\sigma = q / A$. Hence, the equation:

$C(x,t)=f(x2/Dt)$

represents diffusion in a tube by reservoir, though other consequences occur by Ohm's law:

$(I=\frac{V}{R})$

Influence of internal and external sodium on the sodium current-voltage relationship was shown (Landowne and Scruggs, 1981), and the ionic flux is proportional to available ions c(x), and drift speed $J(x) = c(x)\,v_D$, as ion channels explained in synaptic transmission (Sakmann, 2017). The patterns of hydrogen-bonding in pore, especially around pore-lining charged and hydrophilic residues, and around exposed areas of helix backbone were investigated (Smith and Sansom, 1997).

Diffusion criteria were suggested as hydrodynamic argument for weak mass dependence and prediction of a stronger mass dependence (Stokes-Einstein relation or hydrodynamic approach instead of kinetic theory approach) pertain less validity, neither explains weak power-law mass dependence (Bhattacharyya and Bagchi, 2000). Electrostatic forces are incorporated in Nernst–Planck equation (Kirby, 2010). Flux per mole and hence current flux can be converted into Nernst–Planck equation (in amperes per square centimeter):

$I=-(uz\,RT\frac{\partial}{\partial x}+uz^2F\frac{\partial V}{\partial x})$.

The 'Fick's first law' or 'Nernst-Planck-equation' explain it (Su *et al.*, 2017) and osmotic water permeation through a membrane vesicle explains entirely new insights (Su *et al.*, 2017).

Binding caused by diffusion with a binding site that is quite buried can be expressed as: $dxB(t)/dt=-k(t)cxB(t)$ (Berezhkovskii *et al.*, 2011), and e.g. the lidocaine binding with sodium channels and effect of anticonvulsants/ antiarrhythmic drugs explain binding interaction dynamics (Hussain and Backx, 1997). Neither hydroxyl and carbonyl groups in aryl-amine link of lidocaine, were necessary, and hydrogen bonding between structures in the aryl-amine link and the channel were studied (Zamponi and French, 1994). Binding with a certain ligand inside cell can be obtained as revealed (Abel Zur Wiesch *et al.*, 2017).

$$\frac{dAU}{dt} = ku,fAiB - Ku,rAU$$

and represented as (Herrera-Valdez, 2012):

$$v(t) \approx vL + (v_\circ - vL)\,\exp(\frac{gL}{C}t)$$

$IKNeu= -gKNeun4(VNeu-EKNeu)SASyn$

where (KNeu) are the ion channel, E with the KNeu indicates K^+channel reversal-potential, gKNeu as maximum K+ channel conductance, V with Neu is voltage for neuronal membrane & S with Asyn indicates surface-wise synaptic area (Breslin *et al.*, 2018). calcineurin inhibits ANP-induced testosterone production (Henesy *et al.*, 2012):

$$\frac{dfstim}{dt} = k3f\cdot unstim - k4\cdot fstim$$

(fractions of phosphorylated & dephosphorylated receptors, funstim and fstim are fractions of unstimulated activities). Interatomic interactions in hydrogen bonding have been revealed in self-assembly investigation (Ahmadi *et al.*, 2016), and quantum mechanical involvements were implied to study spike protein in SARS-CoV-2 virus (Ching *et al.*, 2021) as:

$AABP(u,v)= \Sigma\alpha Ɛu\Sigma\beta Ɛv P\alpha i,\ \beta j$

The crystal structure of VGSCs reveals the involvement of these channels in initiating the electrical signaling, drug targets and disease mutations though the exact mechanism of voltage dependent activation, selectivity and drug blockage is not clearly known (Payandeh *et al.*, 2011). However, the voltage-gated Na+-channel from Arcobacter butzleri (Na_vAb) indicates interactions in voltage-sensor including unanticipated hydrogen bonds (Payandeh *et al.*, 2011).

The influence of linker size in well-known groups having anticonvulsant activity-4-alkyl-5-aryl-1,2,4-triazole-3-thione derivatives for their affinity for VGSCs was investigated and it was found that hydrogen bonding, desolvation & hydrophobic effects, for 5-[(3-chlorophenyl)ethyl]-4-butyl/hexyl-1,2,4-triazole-3-thiones clarified beneficial sodium channel blocking activity (Paruch *et al.,* 2023).

SODIUM CHANNEL HYDROGEN BONDING (HB)

Selectivity mechanisms

The importance of selectivity filter related hydrogen bonding in C-type inactivation of potassium channels and further insight of the impact of conserved Trp434-Asp447 indole hydrogen bond, it was clarified employing atomic mutagenesis, that Asp447 in Trp434Ind hydrogen bonding goes out towards extracellular area to reduce the local negative electrostatic charge as well as water penetration in the space behind selectivity filter (Lueck *et al.,* 2016).

Since resistance was noted for tetrodotoxin (TTX) to block the bacterial sodium channels Na_VAb, it was found for $Na_V1.4$ by forming hydrogen-bond network at the outer lumen in selectivity filter, and acidic residues just above selectivity filter are crucial for stabilizing the network of hydrogen-bonds between $Na_V1.4$ and TTX (Chen and Chung, 2014).

It was postulated that the H(2)O.Na complex has almost same size as of hydroxylamine and hydrazine cations and the hydrated ion is probably contained there being a part of critical complex between sodium ions and the selectivity mechanism related to sodium channel, and hence, the sodium ions are appropriate for the required hydrogen-bonding provided they contain water of hydration (Hille, 1971).

Some of the main functions comprising solvation, ionic selectivity, and water permeation in the pore region of the VGSCs were studied that uncovered the specific pattern of hydrogen bonding with amino acids for the water motion, though difficult to understand the patterns of a variety of hydrogen bonding for motion in various small states (Singh *et al*., 1996).

Enormous information obtained by nitrogenous cations for cation-selective channels by determining the permeability coefficients (P's) and free-solution mobilities across gallbladder epithelia of rabbit and frog demonstrated the permeation pathway for most of the hydrophilic cations is across tight junctions where the P's increased for the number of available donor protons for hydrogen bond formation and decreased with the molecular size (Moreno and Diamond, 1975). The P's in certain cases fitted by a model of steric restriction if the effect of hydrogen bonding on the size of molecules was considered (Moreno and Diamond, 1975).

Structure & structure-activity relationship

While studying the role of the interaction of azobenzene and p-diaminoazobenzene in the $Na_v1.4$ channel-a voltage gated sodium channel (VGSC) in the inactivated state, it was found that various groups have hydrogen bonding and electrostatic interactions in different binding pockets (Palmisano *et al.,* 2021).

The processes of assembly, activation and inhibition for epithelial sodium channels (ENaCs) were studied using resolution at 3 Å using cryo-electron microscopy that showed the subunit arrangement by complementary hydrogen bonding (Noreng *et al.,* 2020). It is quite interesting to synthesize artificially the molecular assembly of peptides by hydrogen bonding in membrane protein pore structure (Futaki, 1998).

The crystal structure of two alpha-like toxins of scorpion revealed to have high molecular specificity and atomic precision via achieving the backbone geometry, where the C-terminal stretch 58-64 in a pair of main-chain hydrogen bonds between residues 10 and 64 forming a unique tertiary setup (He *et al.,* 1999).

Action of decarbamoyloxysaxitoxin (dcSTX) and decarbamoylneosaxitoxin (doSTX) in frog skeletal muscle fiber showed quite identical activity of saxitoxin (STX) and tetrodotoxin (TTX) for common sharing of four hydrogen-bonding sites and one ion-pair site, as loss of two hydrogen bonds (at C-13 -OH of dcSTX, and amino group in the carbamoyl function of STX) associated with the decrease in potency (Yang *et al.,* 1992).

A study was conducted to understand the relationship of structure and activity for the effect of metoclopramide and related compounds upon surface ionization in fumed silica and it was demonstrated that the formation of hydrogen bonds with neutral silanols occurred from carbonyl oxygens of benzamide (Buyuktimkin and Wurster, 2015).

SODIUM CHANNEL HYDROGEN BONDING IN EPILEPSY

Selectivity for sodium:

Considering the clinical studies, Tyr1537 (S2) and Trp1538 (S2) side chains are recognized as major determinant factors for isoform-selectivity against Nav1.7 were Tyr1537 (S2) & Trp1538 (S2) side chains (McCormack *et al.,* 2013).

For designing the selective VGSC antagonists, the highly conserved residue Asn1540 in human VGSCs was considered highly necessary for binding with anionic aryl sulfonamide part of the antagonist into the anion-binding pocket and the residues in S2 and S3 helices were important determinants where membrane phospholipids modulate the channel activity and drug functions (Ahuja *et al.,* 2015).

Structural modification:

When the terminal carboxamide function was modified by replacing it with a p-NO2 phenyl group (Pandeya *et al.,* 1999), antiepileptic activity with an appropriate dose was obtained in MES test. Structural modification of primary amino group of arylsemicarbazones was not found important (Dimmock *et al.,* 1996), and rather its replacement by aryl ring stopped the activity and a better with the terminal phenyl amino activity that was based on the appearance of the effect of hydrogen bonding

The significant value of the hydrogen bonding of semicarbazono linker is evident (Pandeya *et al.,* 2003) since replacing CONH- with –O-CH2– provided clear information that ScPTZ and MES both tests showed anticonvulsant activity for compounds with –CONH–, but no activity for compounds with –O-CH2–. Furthermore, inactive compounds were formed while substituting carboxamido hydrogen with ethyl group (Pandeya *et al.,* 2003).

Anticonvulsants, receptor-ligand interaction & epilepsy models:

It was explained in review studies by maximum electroshock seizure (MES) models that the anticonvulsant activity of semicarbazones is linked with its protective index higher than that for carbamazepine, phenytoin and valproic acid influencing the binding mainly due to its hydrogen bonding inhibiting the voltage-gated sodium channels (VGSCs) (Pandeya, 2012; Ahsan, 2013) and GABA-transaminase (Pandeya, 2012).

Subcutaneous seizure models including pentylenetetrazole (PTZ), strychnine, picrotoxin and bicuculline have also shown the sodium channel blocking property of other semicarbazones, and it was found that these active anticonvulsant compounds present a hydrogen bonding domain (HBD) and NO_2, Cl, F, and Br substituents in aryl hydrophobic pocket (HP) (Pandeya, 2012).

It was revealed that *s*-triazole ring-3 & 4 form pi-cation interaction with guanidinium group with arginine (Arg1608) of R4 on the voltage-sensing helix S4, and considering the electrophysiological findings showing the molecular foundations of the charge moving via voltage-gated sodium channels (VGSCs) (Yang *et al.,* 1996), the interaction of *s*-triazole is involved in the dependence of 3 & 4 inhibition in view of R4 gating charge to extracellular side of the voltage sensor domain (VSD) during depolarization phase.

The Pandeya model (Pandeya *et al.,* 1999; Pandeya *et al*., 2003) studied the incorporation of aryl/alkyl/alicyclic parts in the carboxamide amino function (Pandeya *et al*., 2000), and the antiepileptic activity of p-chlorophenyl/p-nitro-phenyl/p-bromophenyl substituted semicarbazones was compared with drugs (Pandeya *et al.,* 2002; Riss *et al*., 2008) diazepam nitrazepam and bromazepam and clonazepam.

Influence of linker elongation on human VGSC affinity and antiepileptic Activity in 4-alkyl-5-aryl-1,2,4-triazole-3-thione derivatives was investigated employing structure–activity relationship (SAR) and docking simulations of the interactions between ligands and binding site of VGSC. The beneficial VGSC blocking activity was evident by the HYDE (HYdrogen bond and DEhydration energies) docking scores that provided information about the hydrogen bonding, desolvation, and hydrophobic effects for 5-[(3-chlorophenyl)ethyl]-4-butyl/hexyl-1,2,4-triazole-3-thiones (Paruch *et al.,* 2023).

The HYDE scoring explains the increased receptor-ligand interactions with the elongation of the N4 alkyl chain from butyl to hexyl as shown in a clinical study employing 1,2,4-triazole-3-thione derivative-TP-315 (Makuch-Kocka *et al.,* 2021). The anticonvulsant efficacy of 1,2,4-triazole derivatives was examined by in vivo model of tonic-clonic seizures in mice (Castel-Branco *et al.,* 2009).

The VGSCs affinity was thought as weaker in those compounds having ethylene linker than those with $–CH_2–$ linker, but MES test showed that compounds having ethylene linker was even weaker in antiepileptic activity than that of 5-(3-chlorobenzyl)-4-butyl/hexyl-1,2,4-triazole-3-thiones in the MES test (Kaproń *et al.,* 2019). Absence of hydrogen bonding in phenacylhydrazones expressed no activity (Pandeya *et al.,* 2001).

Several models were presented including the pharmacophore model (Malawaska and Scatturin, 2003) wherein the synthesis of new antiepileptic drugs was assessed for their efficacy.It was initially suggested for the model as auxiliary binding region surrounding the aryl binding-site, whereas a semicarbazone handle aligned with the hydrogen bonding site (Dimmock *et al.,* 1996).

However, the view was modified on the basis of studies carried out on aryl(oxy)arylsemicarbazones having anticonvulsant activity. Nearly coplanar proximal rings were essential for hydrogen bonding (Dimmock *et al*., 1995). The distal binding site as distal aryl ring, aryl binding site as proximal aryl ring, semicarbazono handle as H-bonding area, and auxiliary binding area were presented (Dimmock *et al.,* 1996).

Molecular & biophysical mechanisms:

While investigating the potential genes responsible for febrile seizures (FSs) and generalized epilepsy with febrile seizures plus (GEFS+) and testing mutations by direct sequencing, a microsatellite marker analysis was employed considering the role of already investigated SCN1B gene in the GEFS+ syndrome. The SCN1B gene showed a novel mutation changing an arginine residue with leucine at position 125 (R125L) that was suggested to affect the protein structure/ stability by loss of hydrogen bonding (Fendri-Kriaa *et al*., 2011).

The hydrogen bind interactions were between: N2 acceptor of *s*-triazole ring 3 & 4 and donor C2 amino acid made by Arg1605-R3 gating charge in the front of anion binding pocket; N1of *s*-triazole ring 3&4 and ε-nitrogen of the terminal guanidinium group of arginine Arg1602-R2 gating charge; NH donor group at N2 position in *s*-triazole ring and oxygen atom of carboxylic group in Arg1602 (Paruch *et al.*, 2023).

Brain GABA levels increased and GABA transaminase enzyme both *in vitro* and *in vivo* inhibited by the effect of N^1-(2, 6-dimethylphenyl)-N^4-(2-hydroxybenzaldehyde) semicarbazone (Yogeeswari *et al.*, 2005b). However, it was also noted that medulla oblongata showed increased levels of GABA of the control under the effect of N-(4-ethoxyphenyl)-N^4-(2-hydroxyacetophenone) semicarbazone (Yogeeswari *et al.*, 2005a).

Another study found that GABA increased ten times as compared to that in control under the effect of 4-(2,4-dimethylphenyl)-5-(4-nitrophenyl)-2H-1,2,4—triazole and other cyclized semicarbazones (Mehta *et al.*, 2009), and the activity of aryl-semicarbazones occurred via GABA involvement (Yogeeswari *et al.*, 2003).

CONCLUSION

Molecular and biophysical mechanisms of epilepsies are required to be identified since epilepsy is a complex neurological disorder or a syndrome and has no effective antiseizure treatment yet for several of the its manifestations/ disorders. Hence, receptor-drug studies with three-dimensional geometry are needed to be designed to understand the role intramolecular and intermolecular interactions including hydrogen bonding.

The hydrogen bonds are quite fundamental for understanding the physiophysics of biomolecules at ultrahigh and true-atomic-resolution. Therefore, the present review is a future direction for conducting further studies using X-ray diffraction patterns and a variety of other molecular physiology and biophysics techniques to unravel the mystery of interactions in epilepsy disorders.

DEDICATION

The author dedicates this article to one of his closest friends **Prof. Ferid Murad** (MD, PhD), who passed away last year (September 4, 2023). Ferid Murad was a **Nobel Prize winner in Physiology or Medicine (1998),** and a world fame US scientist (originally from Albania) who began his academic career at the University of Virginia. Dr Ferid and two fellow Nobel Laureates discovered the unknown signaling process associated with nitroglycerin and related drugs releasing nitric oxide, and relaxing smooth muscle by increasing the intracellular cyclic GMP. The author once requested Dr Ferid Murad in 2009 to be a co-director for the proposed Institute of Physiology at the University of Karachi (U of K) under the proposed directorship of one of the mentors of the author at U of K. He accepted the offer happily and was highly generous for arranging a huge financial help for the institute; but it was sad that the plan for the institute could not be implemented.

Prof. Ferid Murad (MD, PhD)

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