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An Overview onPharmacological study and Therapeutic use of Anandamide

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ABSTRACT: Exogenous cannabinoids have long been identified as agents that relieve pain by activation of a G-protein-coupled receptor. But the beneficial pharmacological properties of exogenous cannabinoids (i.e. Δ^9-Tetrahydrocannabinol) such as relieving pain and spasticity are always encountered with untoward effects such as hypomotility, hypothermia and catalepsy. Besides searching for its potential therapeutic application as next-generation therapeutics, anandamide does have various physiological roles in maintaining body homeostasis. In this review, various physiological effects of anandamide and possibilities of its therapeutic intervention have been put forward at a molecular basis.

Keywords:-Endocannabinoids; Anandamide; N-acetyl Transferase (NAT); CB-receptors; NAPE-PLD; NAAA; MAGL; FAAH; FABP; PPAR-α; click-modified chemistry; Si-RNA; Azide-modified Si-RNA

I. INTRODUCTION

In early 1990's, concomitant with searches for exogenous cannabinoids, researches were carried out for endogenous ligands of CB1 and CB2 receptors in the porcine brain. Subsequent identification of two fatty acid neurotransmitters N-arachidonoylethanolamine (anandamide) and 2-arachidonoylglycerol(2-AG) acting as a natural ligands for endocannabinoid receptors led to the notion that endocannabinoid receptors(CB1) are subclass of GPCR receptors that recognizes lipid as its natural ligands. Concomitant to this notion, the subclass of GPCR receptor superfamily have been identified as erg receptors that also binds with lipids such as lysophosphatidic acid and sphingosine1-phosphate.(1)(2)Anandamide is a class of compound (N-(polyunsaturated fatty acyl) ethanolamine that is derived from arachidonic acid and has a role as a neurotransmitter, a vasodilator agent and a serum metabolite. It is a highly potent endogenous agonist of cannabinoid CB1 and CB2 receptors. All endogenous cannabinoids that are identified are derivatives (amides, esters and even ethers) of polyunsaturated fatty acids especially arachidonic acid.

I. BIOSYNTHESIS

N-Acyl-Ethanolamine seems to have originated from an unusual class of triacylated lipids termed N-acyl Phosphatidylethanolamine (NAPE). The conversion of NAPE to NAEs takes place and occurs via many pathways among which three pathways are focussed

Direct pathway:-Anandamide and its N-acyl-ethanolamine congeners are produced from NAPE by a phosphodiesterase of phospholipase-D type in animal tissues such as NAPE-PLD.(3)

Two Step Synthesis pathway:-Hydrolysis of NAPE to N-acyl-lyso PE by Phospholipase (PLA1/PLA2) and the release of N-acyl-ethanolamine from N-acyl lysophosphatidyle thanolamine by lysophospholipase D (lysoPLD).

Phospholipase C-mediated pathway**:** -It is a biosynthetic pathway of anandamide and produced inside the macrophages. The production of anandamide has been implicated in hypotension in septic shock and liver cirrhosis. LPS (lipopolysaccharides) induced production of anandamide in macrophages is exclusively mediated by phospholipase-C/phosphatase pathway and have been seen in RAW264.7 macrophages that exclusively utilizes PLC catalysed cleavage of N-arachidonoylPhosphatidylethanolamine (NAPE) to generate a phosphoanandamide, which is subsequently dephosphorylated by phosphatase like PTPN22(Protein tyrosine phosphatase).(4) LPS mediated anandamide production upregulate PLC (Phospholipase-C) and downregulate NAPE-PLD acts as a salvage pathway when phospholipase-C/phosphatase pathway is depleted. This also indicates that a production of anandamide by PLC/phosphatase pathway can be exploited for therapeutic intervention in autoimmune diseases.

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II. Enzymatic Formation Of Anandamide

N-Acylphosphatidylethanolamine-Hydrolysing Phospholipase-D (NAPE-LD):-It is a 46-kDa protein and suggested to belong to a metallo-ß-lactamase family(8)(9). The member of this superfamily is characterized by a highly conserved motif. In this motif, aspartic acid residues and histidine residues are highly conserved.(4) They are reported to be involved in binding and substrate processing. Zn^{2+} is generally contained in the member of the metallo-ß-lactamase family.(9)However, the exact number of Zn^{2+} ions per molecule has not been elucidated using atomic-absorption spectroscopy. The Distribution of this enzyme in human tissues has been found to be present in the heart maximally followed by brain, testis, kidney, spleen, liver and lung.

N-acyl-transferase (NAT):-NAT is a membrane-protein that can be solubilized in Nonidet P-40 (a complex non- denaturing detergent solubilizing agent used for separating membrane-protein complexes).(4)(5) The optimal PH for the reaction is in the range of 7-10 depending upon the different preparations and different assay conditions. The transaction reaction does not involve high energy ATP molecules. The enzyme activity can be potentially activated by Ca^{2+} and could be replaced by other ions such as Sr^{2+}, Mn^{2+}, and Ba^{2+}(4)(6). The enzyme that catalyzes this reaction chemically abstract the acyl chain from the various phospholipids without itself having any substrate specificity in terms of fatty acid species at the sn-1 position of the acyl donor.(7)

III. STORAGE,TRANSPORT AND UPTAKE AND RECEPTOR INTERACTING PROPERTIES

Unlike any other neurotransmitters that are basically stored in the vesicles prior to be released synaptically, anandamide is produced on demand from plasma membrane when transfer of arachidonic acid from sn-1 position of rare phospholipids to sn-3 position of phosphatidylethanolamine, thus creating N-arachidonoylphosphatidylethanolamine (NAPE). Other pathways include sequential release of arachidonic acid from ethanolamine and phosphatidylethanolamine.

Moreover, cholesterol and ceramides have roles in receptor-dependent and receptor-independent signal pathways of anandamide.(10)Cholesterol behaves as a binding partner for the neurotransmitter mediated by the initial attraction established through hydrogen bond and forming complex with anandamide is attracted more towards the CB1 receptor showing high binding affinity towards the receptor. Cholesterol may regulate in and out of anandamide from CB1receptors by interacting with low affinity cholesterol recognition sites located in transmembrane helices.(10)

The current visualization of plasma membrane as a mosaic of cholesterol-enriched lipid domains bathed in phosphatidylcholine liquid phase led to the importance of lipid rafts and cell function. Lipid rafts are defined as small (10-200nm), heterogeneous, highly dynamic, sterol-sphingolipid enriched domains that compartmentalize cellular processes. Since from chemical point of view AEA is an arachidonic acid derivatives with ethanolamine that added to arachidonic moiety, at physiological pH, the compound no longer poses an ionisable group and the compound consist of one nitrogen and two oxygen atoms that can accept total five hydrogen bonds making the compound slightly more water soluble and it's apolarchain like arachidonic acid displays four double bonds forming a kink in each bond making an angle of 30° with the hydrocarbon axis. (11)(10)

Thus such configuration makes anandamide a compact structure unusual to that of classic hydrocarbon chains. Also the structural flexibility owing to its helical and hairpin conformers altogether makes the compound with dual property of conformational diversity and improved water solubility. Thus,anandamide diffusion through synaptic cleft requires a vehicle.

Despite having poor water solubility, anandamide retains its lipid nature and its binding site is located deep transmembrane domains of the receptor and thus first the molecule have to cross lipid bilayer and cholesterol, by itself play a key role in transport through the membrane along with Some carrier protein.

IV. ENDOCANNABINOID RECEPTORS

The CB1 receptors have been identified as the GPCR receptors most abundantly expressed in CNS with the highest density reaches in the basal ganglia, cerebellum, hippocampus and cortex. It is also present in several presynaptic neurons. Their distribution and their coupling to inhibition of voltage-activated Calcium channel and increasing endocannabinoidformation by increasing intracellular calcium levels makes endocannabinoid system a target tool for modulating the neurotransmitter release.(12)

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In contrast to CB1 receptors, CB2 receptors are more specifically located in immune tissues and cells. Although stimulation of both CB1and CB2 receptors by agonists leads to inactivation and stimulation of many intracellular molecular signalling pathways, and regulation of expression of several genes, the choice between the molecular pathways to be modulated also depends upon the agonist under investigation and cyclic AMP (cAMP). The activation of molecular pathways involve stimulation of heterotrimericGi/o coupled to inhibition of adenylatecyclase (AC) with corresponding inactivation of protein kinase A(PKA) phosphorylation to more complex protein phosphorylation cascades such as phosphoinositide-3-kinase and protein kinase B.(12)(13)

It was also observed that CB1 and CB2 did not mediate all actions of cannabinoids. Such experiments were performed in knockout mice and experiments showed that cannabinoids could still affect blood pressure, pain, inflammation, gastric motility in absence of CB1 and CB2 receptors. This led to the search for new investigations for other receptors.

Some atypical endocannabinoid receptors are:

GPR18:The discovery was made in 2006 that this receptor could be activated by endocannabinoid.The receptor is highly expressed in the spinal cord, small intestine, immune cells,lungs,bone marrow, testis, thymus and cerebellum. It can be activated by anandamide but the principal ligand appears to be N-arachidonyl glycine (NAGly). Receptor activation has propensity to lower blood pressure and can act as a chemoattractant.(14)

GPR55:It is an orphan receptor similar to that of GPR18. It can be activated by anandamide, 2-AG but the principle ligand appears to be putative endocannabinoid called lysophosphatidylinositol (LPI).Physiological expression of this receptor include central nervous systems, adrenal glands, gastrointestinal tract,bladder,kidneys and other wide body tissue distribution. Activation of this receptor leads to hypotension, anti-inflammatory, anti-nociceptive, regulates energy intake and expenditure, and may have a role in osteoporosis. Moreover it is found to have neuroprotective activity against multiple sclerosis.(15)

GPR119:The expression of this receptor is limited to certain tissues like pancreas and gastrointestinal tract and may have a role in energy and metabolism. It is mainly activated by oleoylethanolamine (OEA) and minimal activation with other endocannabinoids like anandamide and 2-AG. Activation of this receptor and regulation of hormones insulin and GLP-1 leads to reduction in food intake, improves in handling of blood sugar and decrease in body weight.(16)

VanilloidReceptors:Transient receptor potential vanilloid (TRPV1) is an ion channel that is primarily expressed in sensory nerves and neurons. It has a protective role in tissue damage and is activated in heat and pro-inflammatory response. Anandamide activates TRPV1.Interestingly; sensory neurons are co-expressed with CB1 making anandamide available to act by modulating and regulating pain signals. This receptor also has a variable role in the brain and anandamide activation reduces pain.

Serotonin Receptors: Among different subtypes of serotonin receptors that mediate a variety of functions, 5-HT3 is unique since it is a ligand gated ion channel. Anandamide directly binds to a 5-HT3 receptor and inhibits its activation. This inhibition is not by the mechanism of blocking but anandamide binds to different sites and by acting as a negative allosteric modulator decreases activation by 5-HT. this inhibition is partly responsible for analgesic action independent of CB1 and CB2 receptors.(17)

Glycine Receptors: This is a ligand- gated ion channel receptor that inhibits nerve activation. The glycine receptors are expressed in spinal interneurons where it modulates pain. Anandamide binds to the allosteric site and increases the activation of this receptor by glycine. Through this receptor, endocannabinoids act at the spinal level and reduces pain.(18)

Peroxisome Proliferator-Activated Receptor (PPAR):The mechanism of action of this receptor is unique since it can directly bind to the DNA sequences and change transcription of genes. Endocannabinoids have preferential selection for the three isoforms PPAR-α, β and γ. PPAR-α is primarily activated by OEA and PEA but anandamide and 2-AG can also activate PPAR-α and γ. The activation of these receptor isoforms has neuroprotection against ischemia, neurodegeneration, reduced nicotine addiction, analgesia, anti-tumour effect, vasorelaxation, weight reduction and reduced inflammation.(19)

IV. IMPORTANCE IN PHYSIOLOGY AND PATHOPHYSIOLOGY OF DISEASES

Reproductive system:Acrosin is the most important protease enzyme that catalyses the

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acrosome reaction. Human sperm have been found to contain CB-1 and CB-2 receptors. Although the effect of endocannabinoid (EC) system is still unexplored, the study of ultrastructural compartmentalization of CB1-R receptor and its stimulation by a stable analog of anandamide i.e., 2-methyl arachidonyl-2'-fluoro-ethyl amide(MET-F-AEA) have shown that the compound mainly resides in sperm, especially in mitochondria and shown a concentration dependent sperm survival. The increase in concentrations from 10nM-1µM of MET-F-AEA has shown to decrease the survival rate. Such a decrease in survival rate have been due to the fact that treatment with MET-F-AEA decreases two prosurvival proteins pBCL2 and pAkt, and increase the expression of pTEN that is the main regulator of PI3K/Akt pathway.(20)

Anandamide also seems to regulate implantation during pregnancy by activation of cannabinoid receptors on the surface of the embryo. Thus, women who smoke marijuana have good reason to quit smoking. There exists a complex network of membrane-associated molecules and signalling such as steroid hormone, growth factors and lipid mediators. The human embryo implant successfully after fully forming a blastocyst. The small cluster of cells called inner cell mass eventually develops into a fetus and the outer layer of the cells convert into trophectoderm. When the cells of the trophectoderm are ready to implant, their metabolic rate increases and they develop a repertoire of surface molecules to adhere to the receptive uterus. The cannabinoid signalling intricately regulates this implantation process that ultimately ends up in events resulting in adhesion of the embryo to the uterine wall.(21)

Central Nervous system:Astrocytes are the cells present in the central nervous system that plays a significant roles in neuroinflammation and production of enzymes such as inducible nitric oxide synthase (iNOS), cyclooxygenase-2(COX-2) and free radical nitric oxide (NO) or proinflammatory cytokines. Endocannabinoids and anandamide itself have the ability to promote anti-inflammatory responses in astrocytes. Experimental studies with a potent and selective anandamide reuptake inhibitor UCM707 showed decrease production of iNOS expressions, tumour necrosis factor (TNF-α) and interleukin-1β.(22)

Alzheimer's disease is a progressive decline in cognitive and memory due to formation of neurofibrillary tangles and formation of β-amyloid plaques that leads to neuronal death. Microglia and astrocyte activation are the main cause of neuritic plaques and onset of inflammatory response in AD. While significant loss of CB1 receptors have been implicated in most part of the brain receptors are increased mainly in activated glial cells and often associated to an overexpression of FAAH in the same cells suggesting an important role of endocannabinoid signalling pathway in modulation of inflammation. Administration of AMT inhibitor VDM11 or of MAGL (monoacylglycerol lipase) inhibitor JZL184, increased extracellular levels of endogenous anandamide and 2-AG respectively. It was beneficial against neurotoxicity by reversing damage in the hippocampus, neuro-inflammation, neurodegeneration and improving long-term plasticity and memory.

Parkinson disease is the second commonest neurodegenerative disorder and is characterized by motor and non-motor symptoms including tremor, bradykinesia,muscular stiffness, loss of postural balance, as well as constipation and depression. It leads to degeneration of dopaminergic neurons in substantianigra and impaired neurotransmission in the basal ganglia. Several mechanisms of damage have been implicated for the pathology of PD and these include oxidative stress, mitochondrial dysfunction, excitotoxicity and neuro-inflammation. Apart from these, a recent study shows the role of the immune system in the autoimmune destruction in the pathogenesis of PD due to formation of antibodies directed towards the PD-associated antigens. Additionally, microglial activation is critical for neuro-inflammation and the production of varieties of inflammatory mediators, such as ROS,cytokines and glutamate that leads to nigral cell death and progression of neurodegenerative processes. It has been observed that the CB1 activity is increased in basal ganglia and hence this receptor is proven to be a therapeutic target for the CB1 agonists to exert a neuroprotective action on dopaminergic neurons in nigral cells. Furthermore, THC and CBD exerted neuroprotective action by reducing denervation of ipsilateralstriatum.Since, following denervation, theeCB becomes overactive, and their anti-inflammatory roles explain their protective role via activation of CB2 receptors on glial cells. JZL184 provided neuroprotective effects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and these effects were unchanged by CB receptor antagonist and recapitulated by COX inhibition which suggest that these effects were not mediated via CB receptors but mediated due to reduction in arachidonic acid and prostaglandins.

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Huntington's disease is a dominant autosomal disorder characterized by loss of movements, emotional problems, and loss of cognition due to mutations in the huntingtin gene. Aberrant aggregation of huntingtin protein leads to cytotoxic effects. The initial damage is evident in the striatum but slowly progresses to other parts of the brain like cerebral cortex, substantianigra,hippocampus, purkinje cells of cerebellum and some parts of hypothalamus and thalamus. HD also leads to increased astrocytes and microglial activation and aberrant expression of huntingtin gene is sufficient to priming and activation of proinflammatoryphenotype.HD is also associated with the altered expression of several elements of eCB systems. Loss of CB1 in neuropeptide Y interneurons and downregulation of CB1 receptors in the basal ganglia and GABAergic neurons respectively contribute to the pathogenesis of HD. CB1 receptors in the glutamatergic neurons seem to be a potentially explored target for endocannabinoids for its neuroprotective action. How cannabinoids can potentially be useful as a drug needs to be investigated thoroughly.

Amyotrophic Lateral Sclerosis (ALS):It is the more common disorder of the motor neurons also known as Lou Gehrig's disease. The neurodegenerative disease affect the motor neuron system of spinal cord, brain stem and progressive atrophy of voluntary muscles leading to difficulty in key movements like chewing,swallowing, talking and breathing at initial stage to complete loss of voluntary control over muscles.90-95% of all cases of ALS are sporadic and the etiology of the disease is still unclear. 5-10% of ALS is genetic and 20 mutations are known to have been associated with the condition. Mutations in Zn/Cu superoxide dismutase (SOD1) constitute almost 20% of all familial cases. Glutamate excitotoxicity, Protein aggregation, mitochondrial dysfunction, axonal deficit and skeletal abnormalities all may be associated with the etiology of the disease. Neuro-inflammationandmisfolded protein aggregation are the hallmarks of the disease. Elevated anandamide and 2-AG levels during ALS-excitotoxic damage likely to play a neuroprotective role. Although the role of cannabinoids is still to be investigated properly, these compounds may exert powerful antioxidant, anti-inflammatory and neuroprotective effects and cause analgesia, muscle relaxation, prolonged neuronal survival and delayed disease progression. The only compound that underwent a completed trial is Dronabinol. The conducted trial consisted of 22 patients that were administered with oral Dronabinol for cramps. The possibility to treat ALS with cannabinoids needs to be further investigated and remains a major challenge for future clinical research.

Cardiovascular system:The role of anandamide in cardiovascular systems can be understood by the ability to induce vasorelaxation in a large number of vascular beds by a number of mechanisms. The effect produced is three-phase response similar to that of tetrahydrocannabinols. The initial phase of the reaction starts with dramatic drop in heart rate and blood pressure that continued for a few seconds and then followed by short hypertensive replay thought to be caused due to vasoconstriction of certain vascular territories, such as the spleen and not caused due to sympathetic stimulation. The third action associated with anandamide is associated with hypotension, bradycardia which persists for 2-10 minutes.(23) The findings are consistent with a metabolically stable and long acting analog of anandamide named R-methyl amide which also produced hypotension and bradycardia. The Experiments also eliminated the possibility of any metabolite indirectly causing such effects.(24)

V. DIFFERENT ENDOGENOUS ENZYMES AS THERAPEUTIC TARGET

Both endocannabinoids and fatty acid ethanolamines have shown to alleviate pain and inflammation, have anti-cancer, anxiolytic, and neuroprotective effects and many of the enzymes that regulate these compounds have been identified as a therapeutic strategy to develop new compounds. These enzymes include FAAH, NAAA,MAGL and COX-2.(25)

NAAA (N-acylethanolamine acid amidase)

Macrophages and peripheral tissues express NAAA in high amount.(26) The catalytic site of human NAAA has been found to possess residues like Cys126, Arg142 and Asp145. Being an aminohydrolase of N-terminal cysteine hydrolase family, the reactive nucleophilic moiety of NAAA, i.e. the thiol group of Cys126 interacts with carbonyl group of the substrate to produce acyl-enzyme adduct. NAAA is highly pH sensitive and recently NAAA inhibitors have been found to have therapeutic effect in pain, inflammation and also have shown to produce PPAR-α-mediated anti- inflammatory effects.(16)(25)(27)

MAGL (Monoacylglycerol lipase)

These enzymes belong to the member of serine hydrolase family.(28) The important

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residues contributing to overall enzyme activity are Ser122-Asp239-His269 catalytic triad and many other residues. Some of the important residues are Cys201, Cys2018, Cys242 and that are hypothesized to stabilize the enzyme.MAGL inhibition increases the 2-AG levels in the brain and since MAGL is responsible for degradation of 2-AG, potential inhibitors can act as a drug target for anti-inflammatory, analgesic and neuroprotective effects.(29)(30)

Fatty Acid Amide Hydrolase(FAAH)

The enzyme belongs to a serine hydrolase family of enzymes first known to breakdown anandamide. Before its discovery it was predicted that membrane associated enzyme was responsible for the breakdown of AEAs and FAAH was identified by Cravatt and co-workers in 1996. Inhibitors of FAAH produce behavioural effects that are significantly different from that produced by the CB1 agonists.

VI. MATERIALS AND METHODS

The following Bioactivity properties of anandamide as a potential drug candidate have been predicted in molsoft Drug-Likeness viewer .

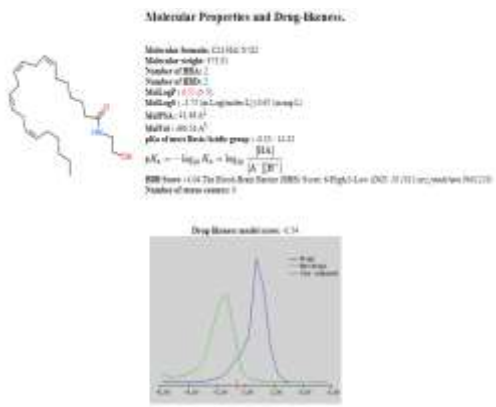

Fig 1: Drug likeness of the anandamide molecule

The PDB file for the FAAH protein has been retrieved from(https://www.rcsb.org/). Anandamide and its target interactions are observed in Autodock.4.0 and the following visualizations are inferred through potential to form hydrogen bonds.

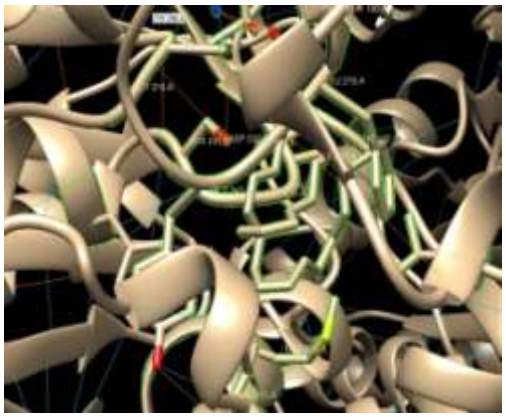

Fig 2: Binding of anandamide molecule in the energy minimized active site Ser241 of FAAH enzyme.

FAAH seems to be a dimeric enzyme that can guide its substrate in and out of the catalytic site by a complex architecture. The substrate reaches the catalytic site via membrane access channel where two charged residues Asp403 and Arg486 allow the entry of the polar head groups of the fatty acid molecules while acyl-binding site contribute to accommodate substrate during catalysis. A solvent- exposed ‘’cytosolic port’’ also accompanies the catalytic site serves to access the enzymes to the cytosolic compartment of the cell.Homodimeric structure of the FAAH, formed from monomers A and B .Embedded in 1-palmitoyl-2-oleoyl-phosphatidylethanolamine (POPE) lipid layer. Anandamide reaches the catalytic site via ‘’membrane access channel’’. Enzyme hydrolysis occurs via two main reaction steps. The acylation and deacylation steps.

During the acylation step, the residue Ser241 nucleophile is activated by the deprotonated Lys142 via electron transfer that also involves Ser217. The activated Ser241 attacks the anandamide carbonyl group leading to the formation of tetrahedral intermediate. Reversal of proton transfer occurs at this point from Lys142 through Ser217, leading to protonation of ethanolamine as a leaving group, thus forming acyl-enzyme adduct. Also a new proton transfer in a catalytic triad also deprotonates nucleophilic water thus forming the final product. The enzyme recycle takes place by restoration of initial protonated state and the enzyme action terminates when ordering of the H-bonding network in the catalytic loop takes place.

The uptake mechanism of anandamide into the cell is unique because its uptake is coupled to its breakdown. Uptake rates are different in cells

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and depend on its correlation with the inherent FAAH concentration. Uptake rates are generally negatively correlated with degree of FAAH inhibition.

II. RESULT

By performing a chemoinformatics analysis of anandamide we came to know about its immense possibility to act as a drug candidate. Being endogenously derived this is less prone to show adverse toxicity and bioavailability and drug-likeness score being -0.54.

III. DISCUSSION

Traditional process of drug discovery based on natural secondary metabolites is slow,labour-intensive and even with advent of combinatorial chemistry and high-throughput screening ,the generation of leads is dependent on reliability of individual reactions to construct new molecular frameworks. Click chemistry is a newer approach to the synthesis of drug chemistry using few practical and reliable reactions.

Click chemistry with alkyne-modified RNA with different receptor ligand azides have been greatly utilised in the preparation of 3'-folate, 3'-cholesterol and new type of anandamide-modified RNA.Anandamide- modified RNA have shown to have surprisingly high transfection properties and opens up the possibility to transfection SiRNA- based silencing gene delivery to neuronal and immune cells. The modified delivery strategy has been shown to transfect even difficultRBL-2H3 cells. Also the silencing effect was shown to have similar effects to that of cationic, benchmark reagent in BJAB human immune cells. Also anandamide-conjugates are found to be non-toxic in nature.

Although unmodified siRNAs possess similar chemical nature to mRNA, they are feasible to attack by several ubiquitous ribonucleases. Moreover, owing to their hydrophilic property they do not bind with the serum proteins and circulate throughout the body and they are excreted quickly by renal excretion exhibiting plasma half-life<10 mind. Thus for aforementioned reasons unmodified siRNAs is a problematic macromolecule drug that has a little "drug-like", perspective.

One of the problems that impedes siRNA delivery is that they are large (~14KDa), high hydrophilic molecules and polyanionic (~42 negatively charged phosphates) molecules that possess intrinsically poor pharmacological properties. For biotechnological application, siRNA molecules are prepared and administered.

Recently, receptor- mediated endocytosis have become an alternative strategy of delivery of siRNA apart from conventional nanoparticles, liposomes and polycationpolymers.Currently the strategy is more successfully implemented with cholesterol-modified RNA but for targeting the neuronal and immune cells that have been found to possess cannabinoid receptors can be targeted using anandamide-modified siRNA despite some uncertainty exist regardingcannabinoid-mediated reuptake.

IV. CONCLUSION

Anandamide is an endocannabinoid molecule that can be of great importance in designing new therapeutics. It has an immense role in physiology and pharmacology and has also been used for many biotechnological applications.

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