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Original Article

A REVIEW ON THE PHARMACOLOGY AND TOXICOLOGY OF ACETYLCHOLINE RECEPTORS AT THE NEUROMUSCULAR JUNCTION

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ABSTRACT

The pharmacodynamics profile of a drug plays an important role in the development of a novel therapeutic agent. The above mentioned basic foundation is also applicable to the development of drugs which are directed towards the neuromuscular junction. The nicotinic acetyl choline receptor has been predominant role at the neuromuscular junction and has minor role in the central nervous system. The nicotinic receptor acts as an important link in the transmission of information from the skeletal muscle to the spinal motor neurons and any disruption of its functioning as caused by various Venom toxins as of curare toxin and mutations as in congenital defect and the autoantibody release as in myasthenia gravis. The easy access and the homogeneous nature of the receptor have helped the scientists to find the pharmacology and the electrophysiological properties. Hence the pharmacological and toxicological studies are the core evidence of a successful drug.

Keywords: Neuromuscular junction, Nicotine, Myasthenia gravis, Toxicology.

INTRODUCTION

The cholinergic transmission in the human body was first explained by Reid Hunt in 1990 [1]. Hunt had carried out the experiment on adrenal gland and found out that there was lowering of blood pressure instead of rising due to the potent Acetylcholine activity which had a potent activity index of 100000 times when compared to Choline [1]. Dale in 1914 demonstrated the pharmacological properties of Acetylcholine and found out two types of receptors as muscarinic receptors and nicotinic receptors [1]. Muscarinic activity mostly related to the activation of parasympathetic nerve endings [1]. The nicotinic acetyl choline receptor is related to the activity of acetyl choline interacting with the sympathetic and parasympathetic nerve endings, the secreting cells of the adrenal medulla and the endplate potential due to activation of the motor end plate of the voluntary muscle [1].

Nicotinic acetyl choline receptor

The nicotinic acetyl choline receptor mainly divided into three subclasses based on the role to be carried forward, the first being the skeletal muscle which is designated to the Neuromuscular Junction, second being the ganglionic class which produces transmission in the sympathetic and the parasympathetic nerve endings and the third being the CNS class confined to the brain and other central areas and are ought to be heterogeneous in the role and molecular composition [1]. The nicotinic acetyl choline receptor belongs to the class of the ligand gated ion channels [2]. The Nicotinic acetyl choline receptor binds to the acetyl choline molecule and causes the protein core to open thus producing conformational changes in the nicotinic receptor [2]. The conformational change causes an influx of sodium ions and the outflow of potassium ions thus resulting in the depolarized state [2]. The net depolarized state produces an action potential or contraction in the nerve ending or results in the release of the transmitter [2]. The channel opens for a millisecond interval and on exposure to various nicotinic receptor agonists for seconds to minute interval produces desensitized state of the receptor producing closed channel [2]. There is also access to calcium facilitating the release of the transmitter, triggering of the signal cascades producing gene regulation and cause activation of the ion channels [2].

Nicotinic receptors- the memebers of gene superfamily

"Karlin and Akabas indicated that the acetyl choline receptors at NMJ are members of the superfamily of the homologous ligand gated ion channel receptors with the composition of different homologous subunits comprising of inhibitory neurotransmitters as Glycine and GABA and the excitatory transmitters as 5-HT_3 receptor [3]. These receptors are formed by gene duplication process [2]. The receptor is composed of central core protein with five subunits surrounded like a barrel staves [2]. The binding of the channel with the neurotransmitter causes it to open the ion channel with conformational change thus produces depolarization [2]. There has been evidence of conversion of the excitatory cation selective ion channel to inhibitory anion selective ion channel due to the change in the three amino acid homology in every subunit of the nicotinic acetyl choline receptor [2].

Subtypes of nicotinic receptors

"Lindstrom stated that the nicotinic acetyl choline receptor is comprised of various subtypes each comprising of different organization of subunits having one central core protein ion channel with five subunits arranged around it [2]. In the homomeric nicotinic acetyl choline receptor, the binding sites for the receptor has been formed by the five subunits between the Interfaces whereas in the heteromeric type, there are two binding sites for the receptor at the interfaces designated as for the alpha subunit as plus side and the minus side for the adjacent subunit [2].

The nicotinic acetyl choline receptor, the immature ones are placed inside and outside of the neuromuscular junctions open and close slowly and have good turn over period [2]. Heteromeric acetyl choline receptor is made of combination of α_2, α_3, α_4, α_6 with that of β_2 or β_4 [2]. The nicotinic receptors comprise of different types of subunits from three to four [2]. The brain has the $(\alpha_3)_2$ $(\beta_4)_2$ α_5 type of subunit composition [2]. The α_5 subunit does not have the capability to form the acetyl choline binding sites like the other α subunits and the β_3 subunit in the same way is not able to form the acetyl choline binding site and thus made it evident that these α_5 and β_3 subunits combine together and make the binding site like the β_1 for the muscle nicotinic receptor [2]. α_4 has been found to have greater sensitivity towards nicotine whereas α_3 has been found to largely exist in the autonomic ganglion but to a lesser extent in the brain [2]. The α_4, β_2, β_4, $\alpha_5$and α_7 are expressed well within the ganglia and also homomeric mixture of α_3 and the heteromeric mixture of α_7 [2]. Desensitization of the nicotinic receptor occurs fast with the α_3 and β_2 combination when compared to that of α_3 and β_4[2]. The α_5 presence as that of the α_3 nicotinic receptor has greater access to calcium and increases the permeability factor and desensitivity power and also the potent nature and the efficacy nature of the subtype [2]. The α_6 subtype of the nicotinic receptor is

very important and present in minor amount in the retina and the catecholamine neurons such as the ventral tegmental area and the substantianiagra and the locus cereleus[2]. α_6 can combine with other α subunits such as α_3 and α_4 and also with β subunits like β_3[2]. The combination of the two subunits like β_2 and α_6 will not form potent nicotinic functional receptors [2]. Agonists produced are only partial active at the α_6 nicotinic receptor [2]. Homomeric (α_7, α_8, α_9 subtypes) nicotinic acetyl choline receptor not like the heteromeric nicotinic receptors is competitively being inhibited by the Venom Toxins of various cobras [2].$\alpha_4$and β_2 has been studied in the calyx of the chicken and evident of its presence in the brain along with the α_3 subtype [2]. Heteromeric (α_7 and α_8 subtypes) have been studied with α_8 found in the cochlea hair along with α_{10} [2]. The subtypes from α_7 to α_{10} have been found to have greater access permeability characteristic feature to calcium and the desensitivity characteristic which might be due to the presence of nearly 5 binding sites of acetyl choline in the receptor [2]. Activation may result due to binding of any sites and the formation of ligand may increase the desensitivity power [2].

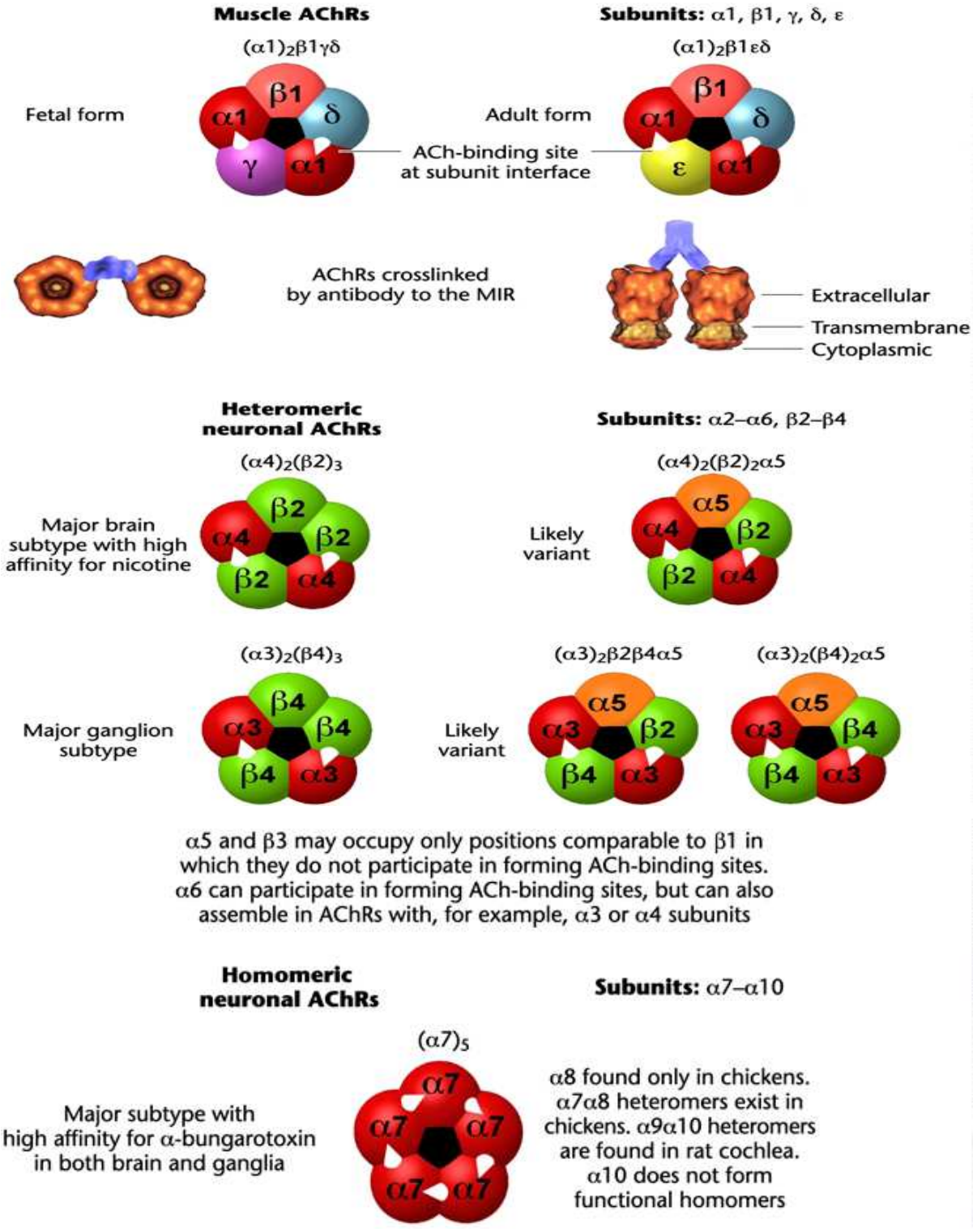

Fig. 1: The figure states the nicotinic receptor subtypes [4].

Structure of Receptor Subunits

The nicotinic receptor has an extracellular domain subunit of 220 amino acids in length with a disulfide like bridge between 128 and 142 cysteine residues [2]. There is evidence of N glycosylation sites present in the extracellular domain [2]. There are transmembrane domains with tightly packed conserved and helix in nature M_1 to M_3 extending from the extracellular domain to the cytoplasm domain [2]. Another transmembrane M_4 domain extends from the cytoplasm domain to the short extracellular domain C terminal tail of 20 amino acids [2]. The subunit composed of the N- terminal of M_1 and other side of M_2 [2]. The cytoplasm domain is the variable region in the subunits sequence from 110 to 127 amino acids [2]. α subunit is designated by the disulfide link cysteine homologues 192 to 193 of the α_1 subunits [2]. These are tend to form one of the three loops of the extracellular domain to form the acetyl choline binding sites [5] and the rest of the binding site is formed by (γ), (δ), (ε), (β_2), (β_4), and (α_7) subunits at the interface with α subunits [2].

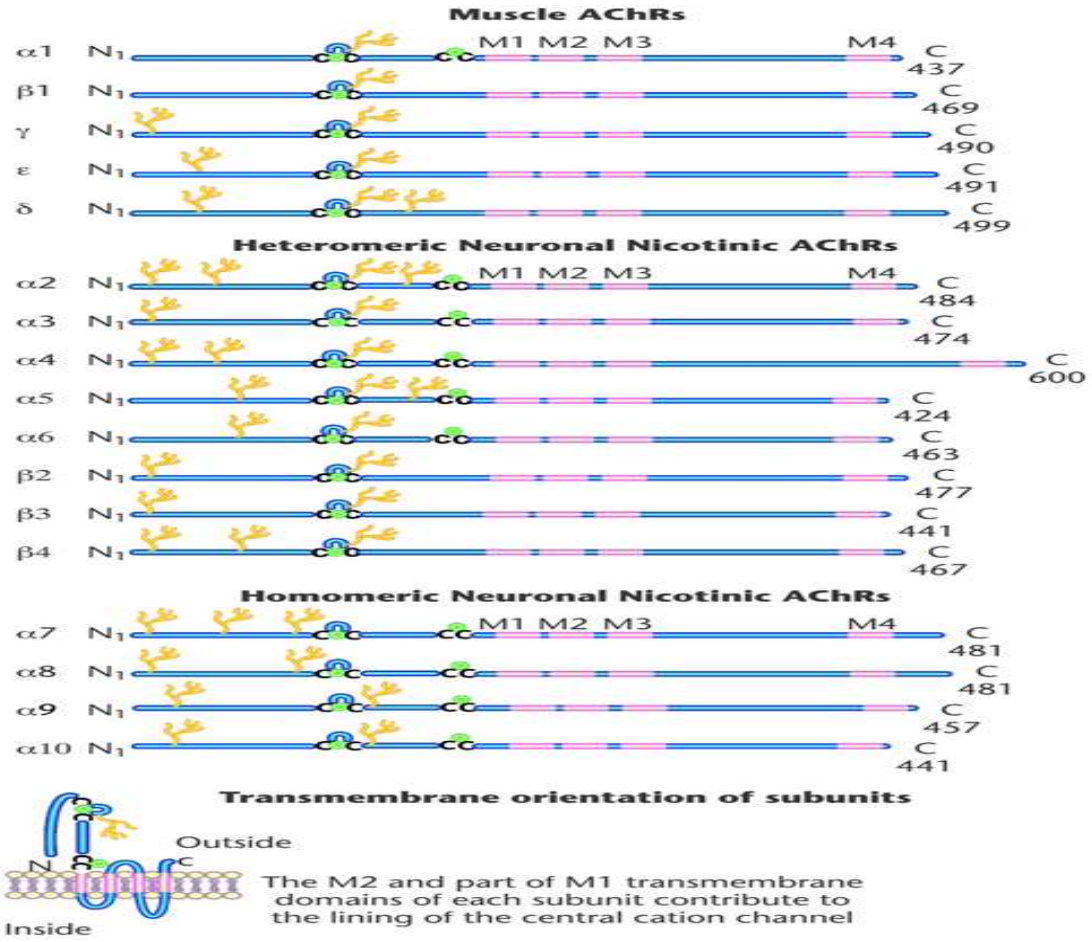

Fig. 2: The figure shows the homologous nicotinic acetyl choline receptor [4].

Functional Roles of Nicotinic Receptor

Pre, post and extrasynaptic receptors

The α_1 subtype of nicotinic receptor has been profoundly involved post synaptically in the transmission at the neuromuscular junction [2]. The α_3 present in the autonomic ganglia plays a post synaptic role in the transmission at ganglionic synapses, with the presence of peri synaptic α_7 receptor developmental changes will take place with transmission in the chicken ciliary ganglia [6]. There has been evidence of α_7 to act both pre and post synaptically in brain [7]. The synaptosomal neurons have been found to regulate the release of various neurotransmitters [2]. There has been no evidence for regulation of transmitters due to release of acetyl choline on the neuronal endings or by less localized release or due to combination of the two phenomenon's [2]. There is no specific localization of the receptor subtype as the same receptor may sometimes act pre, post or peri-synaptically

Knockout and knockin mice

There has been much evidence found about the functional properties of the nicotinic receptor as expressed in the XENOPUS LAEVIS OOCYTES [2]. The disruption of the receptor genes known as Knockouts and the replacement with mutants with altered properties known as Knockins has provided insight to study the functional roles in various animals [2]. The experiment has its own limitation, animals without the sub unit composition may grow and the induction of other subunits may thus cause loss [2]. Also there has been reported that the expression of the multiple nicotinic receptor may produce no compensation in the other sub units [2]. In the Knockin experiment the replacement of normal sub unit with hyperactive mutated sub unit may cause excitotoxicity and change the functional role of sub unit [2]. There has been evidence of death in mice because of lack of α_3 sub unit due to change in the transmission at autonomic synapses and improper functioning of the bladder [9]. There is evidence of maintaining the transmission at the autonomic synapses with the loss of either β_2 or β_4 and not both which would result in the complete breakdown of the system [2].

Knockout of the sub units like β_2 and α_4 result in lots of complications like loss in the nicotine binding in the brain, antinociceptive activity induce by nicotine is lost [10]. $\beta_2$knockout leads to disruption in the behavioral learning and stress hormone development with increase in age [2]. The knockin of α_4 is found to

be lethal until change in expression with Parkinsonian disease along with change in hyperactivity states at the motor synapses and increase in the anxiousness levels [2]. The mutated α_7 produces high lethality in the homozygous mice with apoptosis cell death in the somatic cortex due to high flow of Ca^{+2} ions [2]. With the lack of α_9 it was evident of disruption of the hairy cochlea cell reduction in response produces by the olivery cochlea [2].

Neuronal nicotinic receptors in non neuronal tissues

There has been evidence of the presence of the nicotinic receptor in the various non-neuronal tissues as of the subunits like α_3 and α_{10} in lymphocytic cells, α_3 and α_7 in the bronchia epithelial cell as well as in the tumor cells of the lungs, acute levels of α_7 in the astrocytic cells of brain in the regulation of the Ca^{+2} ions flow at the glial cell [2]. It has also been determined the presence of α_3 and α_7 in keratinocytic cell skin indicating its cytotransmitting behavior [2].

Toxicology Of Nicotinic Receptors At Neuromuscular Junction

Myasthenia gravis

The disease is characterized by weakness in the muscle as well as fatigabile nature [2]. Lindstrom stated that the cause is due to the antibody mediated autoimmune response to the muscle acetyl choline receptor [2]. There has been no immune suppression therapy yet found for this disease [2]. Later monoclonality of antibodies which were used as auto antibodies played a major role to study the characteristic feature of muscle acetyl choline receptor [2]. It is predominant for the antibody to the highly immunogenic region in the auto immune activity [2]. The monoclonal antibodies interact with the sequence of 66 to 76 of the α_1 subunit located around the central axis [2].

Congenital myasthenic syndrome

Engel in 1999 stated that mutation in the order more than 60 in the α_1 sub unit as well as mutation in acetyl choline esterase molecule has been major cause for myasthenic syndrome, thus making α_1 a predominant cause for this syndrome [11]. The mutations that tend to lower or increase the acetyl choline affinity are lethal acting as well as mutation with increase in the choline potency for opening of the channel or inhibition of the functional activity [2]. There has been a pathological disorder related to the study involved in the mutated species in the cell with clone sub unit and also studies involving electron microscopical and electro physiological in people [2].

Epilepsy

There has been evidence of autosomal dominant nocturnal frontal lobe epileptic condition due to mutations of α_4 and β_2 sub unit in the M_2 transmembrane region [2]. The α_4 and β_2 sub unit are actively involved in the release of the GABA neurotransmitter and due to the mutation causes reduced cortical inhibitory functional activity at the α_4 and β_2 pre synaptic receptor [2].

Dysautonomias

There has been evidence of the para neoplastic dysautonomia due to the production of auto antibodies to α_3 sub unit of receptor causing impairment in the transmissive behavior at the ganglia of autonomic synapses [2].

Alzheimer's Disease

There has been evidence of minor involvement of the nicotinic receptor (α_4 and β_2) in the disease but to a major extent the dementia is caused due to formation of plaques and the tangles in the neurofibrial region [2]. There has been medication developed as acetyl choline esterase inhibitors with effective results and the nicotine having neuroprotective effects along with the addictive effect [2].

Parkinsonism Disease

There has been evidence of the minor involvement, due to the loss of nicotinic receptor and also the dopaminergic loss of neurons thus causing tremors, rigidity and predominantly affecting the motor behavior [2]. Nicotine and Nicotine agonists has been proved to be effective in such a state as it increases the release of dopamine both pre and post synaptically [2].

Schizophrenia

The schizophrenic state is characterized by various symptoms like delusion, hallucination and bizarre behavior [2]. It has been found that α_7 deficiency in the receptor may be the lead cause [2]. Heavy smoking may increase the level of α_7, α_4 and β_2 levels it was found that there was not such rise in the levels in chain smokers and levels kept reduced and normal [2]. It has also been found as the lack of effect in gating mechanism thus enable to filter out the irrelevance stimuli [2]. It involves the locus of α_7 and the repair of gating is effective by the use of nicotine [2].

Tourette Syndrome

The syndrome is characterized by various symptoms like the anxiety and obsessive compulsive behavior and the change in motor behavior [2]. There has been effective drug, Haloperidol especially in children to tackle this syndrome and patches of nicotine and nicotinic receptor channel blocking antagonist, Mecamylamine was really very effective [2]. Thus evident that blocking the nicotinic receptor may reduce the release of dopamine and produce a therapeutical outcome [2].

Attention Deficit Hyperactivity Disorder

It is a disorder which is characterized with the following symptoms like distraction and impetuosity and the treatment involves Pemoline, Amphetamine and Methyl phenidate acting through dopamine transmission [2]. Since Nicotine Agonists enhance the cognitive behavior, novel drugs such as 2-Nicotine and ABT-418 have been produced to tackle this disorder [12,13].

Anxiety

2- Nicotine has been expected to cause anxiolytic state and can be confronted with the with drawl induced anxiety state [2]. The anxiolytic state is less marked when compared to benzodiazepines and the nicotine agonists may enhance cognitive state and less impairment of motor function [2].

CONCLUSION

There have been new drugs synthesized in the market for their muscle relaxing activity which are α_1 nicotinic receptor selective agonist and antagonists. The nicotinic transdermal patches and nasal sprayshave been used for cessation of smoking. There have been nicotine agonists in market with a combination of positive and negative effects. The rising blood pressure and heart rate with tumor developmentare the negative effects reported. The positive effect includes antinociceptive, anti-anxiety and neuroprotective activities. There have been indications of the development of new novel drugs with less negative effects as with selective agonists at (α_4 and α_7) units of receptor and neglect the involvement of α_3 as is related with undesirable effects. There has been continued research by the companies in the development of the agonists actively involved in promoting the positive effects at the nicotinic receptor.

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