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Mechanism Based Pharmacotherapeutic Development and Neurochemistry Involved in Nicotine Dependence Induce Withdrawal Syndrome

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ABSTRACT

Nicotine dependence is recidivism condition that requires various neuronal mechanisms to understand. There is a need to explore neurochemistry and mechanisms based on which pharmacotherapies can be developed to avert nicotine dependence. Neurochemical mechanisms involved in nicotine dependence include various nicotine acetylcholine receptors and their subunits like β2, α7, α4β2, α6, α5/β2, α5, α5β4, α4β2. Neuronal nicotinic acetylcholine receptors can regulate nicotine dependence via many pathways throughout the central nervous system . Various pharmacological and non- pharmacological measures individually or in combination are used to prevent nicotine dependence induced withdrawal by acting on their target receptors.

Keywords: Nicotine dependence; Neuronal mechanism; Nicotine acetylcholine receptors; Pharmacological; Non-pharmacological measures

Abbreviations: WHO: World Health Organization; **DSM:** Diagnostic and Statistical Manual of Mental Disorders; **nAChRs:** Nicotinic Acetylcholine Receptors; **NRT:** Nicotine Replacement Therapy; **GABA:** Gamma-Aminobutyric Acid; **DA:** Dopamine; **AMPA:** A-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid; **NMDA:** N-Methyl-D-Aspartate Receptor; **VTA:** Ventral Tegmentum Area; **NAcc:** Nucleus Accumbens; **PFC:** Prefrontal Cortex; **CRF**; Corticotropin-Releasing Factor; **5HT:** 5-Hydroxytryptamine; **NTS:** Nucleus Tractus Solitarius; **PVN:** Hypothalamic Paraventricular Nucleus; **MAO:** Monoamine Oxidase; **EOPs:** Endogenous Opioids; **Hcrt:** Hypocretin; **MPEP**; 2 Methyl -6-(Phenylethynyl)-Pyridine; **Cys:** Cystine

INTRODUCTION

According to world health organization **(WHO)** "drug dependence" means a strong craving to take the addictive substance or drug of abuse. As per the guidelines of Diagnostic and Statistical Manual of Mental Disorders following features must be experienced by the subject: a) Strong craving or urge to take the drug of abuse; b) Recalcitrant behaviour to take addictive substance or drug; c) Continous use of the drug of abuse to attenuate the withdrawal syndrome; d) Continous and prolonged use of a drug to achieve desired result due to tolerance; e) No interest in other pleasurable activities and f) chronic use of drug despite their harmful effects.

Nicotine dependence is characterized by both tolerance and withdrawal symptoms as a result of nicotine use. Nicotine dependence can occur with cigarette smoking, smokeless tobacco use, cigar or pipe use. Physical dependence is characterized by desensitization to elements of nicotine intoxication (tolerance), sensitization to nicotine-induced incentive salience (craving), and withdrawal after pharmacokinetic elimination. As stated in DSMIV-TR, withdrawal symptoms include depressed mood, insomnia, irritability, anxiety, difficulty concentrating, restlessness, and weight gain or increased appetite. The repetitive use of nicotine, compelled by positive reinforcement (pursuit of pleasure) and negative reinforcement (avoidance of withdrawal), also becomes a psychologically conditioned behavior. With time other causes like mood, environment, behaviour and sensations also independently trigger the craving to smoke [1]. Discontinuation and abruption of tobacco intake via cigarette smoking or any other source precipitates affective and somatic withdrawal symptoms which include palpitation, decreased heart rate, anxiety, rigorous craving for nicotine, loss of attentiveness, impatience, depressed mood, restlessness, weight gain and increased appetite [2]. The nicotine induced withdrawal syndrome generates anxiety and discourages person taking large amount of nicotine. To practice restraining oneself from indulging in nicotine which produces drive to relapse? Nicotine is a naturally occurring alkaloid found in the members of the solanaceous plant family such as potato, tomato, green pepper and tobacco. It is a tertiary amine whose levorotatory isomer produces the majority of its physiological effects. This is the main isomeric compound present in tobacco smoke. Nicotine

is a weak base that can readily cross the blood–brain barrier at physiological pH. Nicotine acts as an agonist at the nicotinic acetylcholine receptors (nAChRs) of the cell body of autonomic ganglia, adrenal medulla, neuromuscular junction and brain [3]. Physiological action of nicotine on human body is acute increase in blood pressure and heart rate, although rapid tolerance to these effects develops as nicotine exposure is prolonged. Nicotine also increases circulating catecholamine levels i.e epinephrine which, in turn, reduces body weight. These effects occur at the plasma nicotine concentrations to which smokers are exposed, reaches upon their optimum level. Neuronal nicotinic acetylcholine receptors are ligand-gated ion channels comprising five membrane-straddling subunits that combine to form a functional receptor [4] and include nine isoforms of the neuronal α-subunit (α2–α10) and three isoforms of the neuronal β-subunit (β2–β4). These subunits combine with a stoichiometry of two α and three β, or five α7 subunits to form nAChRs with distinct pharmacologic and kinetic properties. Acetylcholine is the major endogenous neurotransmitter that binds and activates nACh receptors. Many nAChRs are situated on presynaptic terminals and modulate release of various neurotransmitters [5]. Nevertheless, nAChRs also are located at somato dendritic, axonal and postsynaptic sites. Nicotine addiction induced withdrawal syndrome is a complex behavioral phenomenon dependent on several systems, but the main reinforcing effect of nicotine depends on the activation of the mesolimbic dopaminergic system. Administration of the nicotine antagonist like mecamylamine into the cerebral ventricles of the mesolimbic dopamine neurons attenuates both locomotor activating and rewarding effects of nicotine [6]. Owing to the wide distribution of nAChRs, administration of nicotine stimulates the release of most neurotransmitters throughout the brain [7]. Which are involved in the pleasing effects of nicotine? These behavioral abnormalities develop gradually and progressively during a course of repeated and chronic exposure of nicotine, and can persist for months or years after discontinuation. As a result, drug addiction can be considered a form of drug-induced neural plasticity [8]. The stability of the behavioral abnormalities that define nicotine addiction suggests a role for gene expression in this process. Repeated exposure to nicotine alters the gene expression in various specific parts of the brain. Such distorted expression of genes then mediates altered function of neurons and the larger neural circuits within which the neurons operate. Ultimately, such neural circuit changes underlie the behavioral abnormalities seen in nicotine addicts [8-9]. There are various mechanisms that are involved in alteration of gene expression in various parts of the brain related with repetitive use of nicotine. These include altered transcription of genes, altered processing of primary RNA transcripts into mature mRNAs, altered translation of these mRNAs into proteins, altered dispensation of proteins, and altered trafficking of mature proteins to their intracellular sites of action that will lead to release of various neurotransmitters that would play major role in nicotine addiction. Repetitive use of nicotine causes changes in various intracellular signaling pathways at synaptic transmission, which eventually sends signal to the cell nucleus that will alter the transcription of various proteins or transcription factor in various brain regions [10].

ROLE OF NEURONAL NACHRS IN MOLECULAR MECHANISM OF NICOTINE DEPENDENCE

Neuronal nAChRs are ligand-gated cation channels that are activated by the endogenous neurotransmitter acetylcholine **(ACh)** and the exogenous tertiary alkaloid nicotine [11]. nAChRs belongs to super family of Cys-loop ligand-gated ion channels that include receptors for γ-amino butyric acid (GABAA, the GABAB, and GABAC receptor), glycine, and 5-hydroxytryptamine **(5-HT3)** [12]. These ligand gated ion channels have similar structure and function and all the subunit of this family have a pair of disulfide bonded cysteine separated by 13 residues (Cys-loop) in their extracellular amino terminus [13]. nAChRs mediate fast and direct synaptic transmission at neuromuscular junction and autonomic ganglia. These receptors are expressed in neuronal soma where they modulate excitability directly. nAChRs are located in presynaptic terminal where they facilitate calcium dependent release of neurotransmitters like adrenaline, glutamate, dopamine, serotonin by indirect or direct mechanism. In indirect mechanism of nicotine dependence, there is role of sodium influx causing membrane depolarization and voltage gated calcium channel activation [14]. Direct mechanism involved the activation of voltage gated calcium channel or direct influx of calcium at synaptic cleft and non-neuronal transmission of nAChRs leading to nicotine dependence [14]. Previous studies show that repeated exposure of nAChRs to nicotine produce dependence by sensitization [15]. Nicotine dependence is induced by two ways: i). increased pharmacological effect due to increase in the number of nicotinic acetylcholine receptors ii) strengthening their binding with the effector proteins. Binding of nicotine to the nAChRs opens voltage-gated calcium channels by inducing a change in the conformation of α and β subunits, which result in the altered brain concentrations of various neurotransmitters like dopamine, serotonin, noradrenaline, γ aminobutyric acid, glutamate, acetylcholine, and endorphins. Nicotinic cholinergic receptors are widespread in the central and peripheral nervous systems; however, nicotine dependence is particularly affected by receptors localized in the ventral tegmental area, which promote release of dopamine in the nucleus accumbens and prefrontal cortex. The initial pleasing sensations produced by nicotine are positively reinforcing. Prolonged and chronic exposure to nicotine leads to neuroadaptation, a process by which the number of binding sites on the nicotinic cholinergic receptor change contributing to physical dependence i.e. down regulation of receptor [1].

Therefore, Nicotine dependence merits an exhaustive investigation to identify various related and other novel pharmacological approaches to ameliorate the pathological condition of nicotine dependence.

PHARMACOTHERAPIES OF NICOTINE DEPENDENCE

Treatment of nicotine dependence includes:

1. Pharmacological measures
2. Non- pharmacological measures

Pharmacological Measures Includes

The first choice drugs for attenuation of nicotine addiction include bupropion, varenicline, cytosine and nicotine replacement therapy. Second line of drugs which abolish nicotine dependence induced withdrawal symptoms are clonidine and nortriptyline. The therapies currently approved by the US Food and Drug administration **(FDA)** for smoking cessation include:

Nicotine replacement therapy (NRT)

When a person stops nicotine use, NRT acts by relieving the craving and withdrawal symptoms. It has reduced receptor responsiveness to acetylcholine which brings satisfying factor when cigarette is taken by person [16]. People using NRT during quit attempt are likely to have greater chance of success rate by using combination nicotine patch and faster acting form [17- 18].

Nicotinic Agents

Transdermal nicotine patches: These are applied on the skin to deliver nicotine to plasma at steady rate. The transdermal nicotine formulations being marketed currently include NicoDerm, CQ patch, Nicotrol patch & Habitrol patch. They vary in design, pharmacokinetics and duration of wear [19]. The continuous uses of these patches in treatment of nicotine dependence also benefit the characteristic lapse [20].

Gum: It is available to the nicotine addicts as transmucosal delivered nicotine polacrilex (nicotine gum). It is available in two doses: 2 mg and 4 mg. User instructions for its consumers include use a piece of gum every 1 to 2 hours for the first 6 weeks, then to reduce use to one piece every 2 to 4 hours for 3 weeks, and one piece every 4 to 8 hours for 3 weeks. Prolonged use of gum cause gum disease as nicotine constricts blood vessels of gum [19].

Lozenges: The mechanism of action is that it is absorbed slowly through buccal mucosa and then circulated in the blood. It should not be chewed. Studies show that smokers who have taken treatment before with other drugs have good outcome with active lozenges treatment [21].

Nicotine inhalers: Pulmonary nicotine delivery in nicotine inhalers can be maximized by use of nicotine salt 10 mg, of which 4 mg can be delivered and 2 mg is absorbed [22]. This formulation has more physiological pH than pure nicotine where mass of the particle should be optimal for alveolar absorption. Flavoring agents can be added to enhance subject compliance [23].

Vaporizer: It is a device used to release the active ingredients of cannabis or tobacco, by heating the material, ideally to 180°C, due to which the active compounds are converted into aromatic vapours that contain zero particulate matter and less harmful gases such as carbon monoxide. Water pipe is used to filter and cool the vapours, which are, then inhaled directly, through a pipe, for subsequent inhalations. Little smoke is produced at cooler temperatures, through this technique and less material is required to achieve the same smoking effect [22].

Non nicotinic agents

Bupropion: It is a drug that is primarily used as an atypical antidepressant and for smoking cessation by reducing the side effects of withdrawal. Bupropion inhibits neuronal reuptake of both dopamine and noradrenaline. Studies have shown that low doses of buproprion in rats block the rewarding effect of nicotine and reverse the negative effects of nicotine withdrawal [3]. Bupropion also serves to inhibit the nAChR but has biorhythmic side effects such as nausea, headaches and weight loss [24].

Varenicline: It acts through nicotinergic α4β2 acetylcholine receptor that leads to dissemination of the craving for smoking and preventing uptake nicotine from cigarettes. Addicts have reported various side effects like dreams, sleep disturbances, headache, nausea, dizziness, fatigue and gastrointestinal complaints. It is contraindicated for use by pregnant women, children, and adolescents, as well as by smokers with mental illnesses in view of a few reported cases of suicidal thoughts and behavior. It interacts with cimetidine, warfarin, and nicotine replacement drugs [25]. Previous studies have shown that it stimulates α4β2 nAChRs to maintain a moderate level of dopamine release, which reduces cravings and withdrawal symptoms during abstinence from smoking and also blocks the reinforcing effects of nicotine withdrawal [3].

Clonidine: It is α2-adrenergic receptor agonist. It acts on brain to reduce sympathetic neural outflow that results in symptoms like sedation and anxiolysis, as well as potential hypotension, bradycardia, and dry mouth. It brings calming and anxiolytic effects to the subject who is trying to quit smoking. Studies have shown that clonidine reduces the cost and duration of withdrawal processes. Studies have also shown that the success rate in clonidine treated subjects, which is verified by serum clonidine concentration, is more than twice as compared to placebo treated subjects [3]. Common side effects of clonidine are dry mouth, drowsiness, sedation and some allergic reactions.

Nortriptyline: It is a tricyclic antidepressant which acts by inhibiting the reuptake of norepinephrine, serotonin, with negligible effects on dopamine reuptake. It has antagonistic effects at a variety of receptors: Strong: H1, Moderate: 5-HT2, α1-adrenergic, mACh, Weak: 5-HT1. It is used in combination with transdermal nicotine as it shows synergistic effect [19]. Studies show that it improves the somatic signs of nicotine withdrawal in rodents [26]. Side effects linked with it are increase in suicidal thoughts, dry mouth, low vision, constipation and urinary retention due to muscarinic receptor blockage, sedation, weight gain due to histamine receptor inhibition and orthostatic hypotension due to inhibition of α1 adrenergic receptors.

Cannabinoid receptor antagonist; rimonabant: Endocannabinoids are responsible for regulation and synthesis of GABA and dopamine that play an important role in reward, addiction and substance abuse. In nicotine addicts, CB1 receptors are unregulated. Rimonabant acts by selectively blocking the cannabinoid-1 receptors. It decreases nicotine self-administration and other behavioral effects [27].

Nicotine vaccines: It is a vaccine against nicotine which induces antibodies against the nicotine molecule in the body, preventing the drug from reaching neural receptors that produce the smoking like effects [19]. Administration of nicotine vaccine, leads to gradual rise of antibody levels, which may attenuate nicotine withdrawal symptoms, and the possible persistence of the antibodies potentially provides long-term protection preventing relapse [28].

Mecamylamine: It is a drug originally marketed for lowering blood pressure. It is a non selective nAchR antagonist. At high doses it shows adverse effect like drowsiness, hypotension and constipation [22]. It moderates the levels of dopamine and acetylcholine release in brain [27].

Monoamine oxidase (MAO) inhibitors (Moclobemide & Selegiline Hydrochloride): Monoamine oxidase is biological proteins or enzymes that are involved in the catabolism of various neurotransmitters basically dopamine, serotonin and nor-epinephrine. Nicotine dependence leads to inhibition of MAO in brain. MAO is responsible for reinforcing and rewarding effects of nicotine; therefore MAO inhibitors are used for treatment of nicotine dependence. Selegiline is also competitive inhibitor of CYP2A6. Most common side effects of these drugs are dry mouth [29].

GABA Modulating Drugs (Baclofen, Gabapentin, Tiagabine, Vigabatrin): (GABA)-ergic transmission regulate reinforcing effects of nicotine. These drugs act by inhibiting nicotine induced dopamine release in nucleus accumbens. It also inhibits locomotor sensitization induced by chronic exposure of nicotine. Baclofen is found to be GABAB receptor agonist [27]. It inhibits nicotine induced increase of DA in nAc. Gamma Vinyl GABA **(GVG)** is an irreversible inhibitor of GABA transaminase that plays a prominent role in GABA metabolism. GVG attenuates nicotine induced withdrawal syndrome by decreasing the level of dopamine in NAc [27]. Recent developed compounds like GS39783 and BHF177 are novel GABAB receptor-positive allosteric modulators, they inhibits nicotine self-administration in rodents [27].

AMOP-H-OH (6-[5-(azetidin-2-ylmethoxy) pyridin-3-yl]hex-5-yn-1-ol) or sazetidine-A: It is antidepressant drugs that play an important role in modulating neuronal nicotine acetylcholine receptors through its selective partial antagonism or inhibition of nAChR function. Various receptors through which it shows its action are α4β2, α4β4, α3β4 and α1 [30-31].

Ispronicline: It is a selective partial agonist of the central alpha4beta2 neuronal nicotinic acetylcholine receptor **(nAChR)**. It also shows increased cognitive effects in nicotine dependent rats [31-32].

Cytisine: It is a plant alkaloid that acts as a partial agonist of alpha4beta2 nicotinic acetylcholine receptor [33]. It shows its action by moderating levels of dopamine and interference with acetylcholine signaling by modulating various receptor like α4β2 subunits (α3/β4),(α7) nAChRs to show its action. Previous studies have approved the role of cytisine glutamate exchanger in attenuation of nicotine induced withdrawal symptoms [31, 34].

Opioid receptor antagonist (Naltrexone): Opioids receptors can modulate the rewarding and reinforcing effects of nicotine. Naltrexone is endogenous opioid **(EOPs)** receptor antagonists. It acts by blocking release of dopamine through opioid receptors and decrease the aberrant effect of nicotine [35].

Hypocretin/ Orexin (neuropeptide): It is neurotransmitters which function by regulating arousal, wakefulness and appetite [36]. It acst by blocking Hcrt1 receptor **(SB334867)** or both Hcrt-1/2 receptors which are G-protein coupled receptors [37].

Anxiolytic drugs: These drugs are used for the treatment of nicotine addiction, because nicotine has potential to decrease anxiety and stress in addicts. During nicotine withdrawal phase anxiety is most prominent feature. So anti-anxiety drugs like diazepam, buspirone, beta blockers and ondansetron reduce the anxiety linked withdrawal symptoms in subjects [24].

Cytochrome CYP2A6 inhibitors: Cytochrome CYP2A6 enzyme metabolises nicotine into cotinine. Previous studies have shown that by inhibiting CYP2A6 enzyme, level of nicotine remains up regulated in systemic circulation, reducing the craving of smoke. The inhibitors like methoxsalen and tranylcypromine together with NRT are used for the treatment of nicotine addiction [24].

Lobeline: It is a nicotine receptor agonist derived from plant Lobelia inflates, an alkaloid in nature. It was the first drug used for attenuation of nicotine addiction [27].

Dopamine based medication: D3 receptors play a significant role in nicotine reward and addiction. Inhibition of DA transmission in reward circuit of brain acts as anti-nicotine therapy. Partial agonist of dopamine D3 receptor, BP897, dose dependently reduces the nicotine dependence induced withdrawal symptoms in rodents. SB-277011A, a selective D3 receptor antagonist has shown its efficacy in abolishing nicotine seeking behaviour in dependent animal. Nicotine enhances DA clearance in rat NAc, suggesting that nicotine regulates dopamine concentration by the DA transporter **(DAT)**. Nicotinic receptor antagonists DHβE decreases the level of dopamine by modulating the functioning of DAT [27].

Glutamate receptor 2/3 receptor agonists: It blocks the rewarding effects of nicotine by negatively regulating glutamate release at presynaptic site of brain. LY379268 is a potent mGluR2/3 agonist. Administration of LY379268 into VTA or the NAc dose dependently inhibits nicotine self-administration in rodents [38].

mGluR5 receptor antagonist: 2-methyl-6-(phenylethynyl)-pyridine **(MPEP)** is a selective mGluR5 receptor antagonist. Inhibition of mGluR5 receptors by MPEP reduces the nicotine-mediated enhancement of glutamate transmission in the mesolimbic DA system, and therefore attenuating the rewarding effects of nicotine in rodents [39].

N-methyl-D-aspartate receptor antagonists (NMDA): NMDA is the major excitatory neurotransmitter in the brain and plays a decisive role in the nicotine dependence. Memantine, a non-competitive, selective NMDA receptor antagonist, inhibits the acquisition of nicotine self-administration in rodents [40].

Non Pharmacological Measures

Novel therapies

Novel approaches for nicotine dependence is immunization against nicotine. Antibodies induced by vaccine bind nicotine in the blood, thus preventing it from reaching the nicotine receptors in the brain and prevent nicotine addiction. It is safe and generally well tolerated in rodents [19].

Anti-smoking vaccine (Nic VAX)

It is a novel anti-smoking vaccine named as NicVAX32, it is a nicotine conjugate vaccine intended to eliminate physical addiction to nicotine and is presently in clinical trials. NicVAX consists of the hapten 3'-aminomethylnicotine, which has been attached to Pseudomonas aeruginosa exoprotein A. It is approved drug by food and drug administration **(FDA)**. According to National Institute on Drug Abuse, NicVAX can potentially be used to inoculate against addiction [19].

Green smoke electronic cigarette

Green smoke electronic cigarette are healthier than traditional cigarettes and are legal. They produce same palpable sensation and oral fixation that smokers desire, satisfying ones' tobacco cravings as well. They do not burn any tobacco, but rather, when you inhale from an e-cigarette, you activate a "flow censor" which releases a water vapour containing nicotine, propylene glycol, and a scent that simulates the flavor of tobacco. A nicotine-free cigarette: where nicotine has been replaced by black and red pepper capsaicinoids. Coupling this with nicotine patches could provide the ultimate anti-smoking strategy [19].

Behavioral Treatments

Behavioral interventions can play a fundamental role in nicotine addiction treatment. Behavioral methods are employed to (a) find out high-risk relapse situations, (b) create an aversion to smoking, (c) develop self-monitoring of smoking behavior and (d) establish competing coping responses. Avoiding smokers, smoking environments and receiving support from family and friends is also beneficial. Smokers must not only learn behavioral and cognitive tools for relapse prevention but must also be ready to apply those skills in a crisis . Learning and using coping skills are used for both short- and long term prevention of relapse [19].

Diet

Patients who quit smoking tend to gain weight; therefore, encourage patients to follow a low-calorie diet and exercise regimen during and after cessation [19].

Activity

While attempting smoking cessation, exercise has been shown to help curb weight gain and to help alleviate nicotine withdrawal symptoms [19].

Hypnosis

Hypnotism is said to be an excellent aid in helping a user to finally break the habit of smoking [19].

Acupuncture

Acupuncture is a treatment that apparently has no side effects. Laser therapy based on acupuncture principles but without the needles, has also been developed [19].

Motivational Therapies

Motivational books and websites can provide a number of ways to motivate smokers to quit smoking. Some people have been able to find the motivation to quit just by calculating how much money they will save after they quit smoking [19].

Quit meters

Small computer programs that keep track of quit statistics such as amount of "quit-time", cigarettes not smoked, and money saved.

Herbal treatments

Mucus-Clear: Homeopathic remedy clears phlegm and relieve throat congestion, Crave-Rx Drops: Supports mood and well-being while quitting smoking, NicoTonic: Homeopathic remedy relieves the effects of stress, worry and nervous tension, plus supports nervous system health, Rx-Hale: Supports the brain and nervous system while quitting smoking [19].

RECENT PHARMACOLOGICAL INTERVENTIONS USED TO ATTENUATE NICOTINE ADDICTIVE PROPERTIES

Certain pharmacotherapeutic approaches that have been approved by US FDA include nicotine replacement therapy (using nicotine gum, nicotine nasal spray, and transdermal nicotine) and administration of drugs like varenicline and bupropion. These pharmacological treatments available for smoking cessation are associated with symptomatic relief, but with low efficacy and more side effects. Moreover, the existing treatment options fail to inhibit the pathophysiological progression of the nicotine dependence. Therefore, the development of new strategies and treatments is necessary for attenuation of nicotine withdrawal syndrome associated with nicotine addiction (Table 1).

Table 1: Novel therapeutic targets/drugs being explored in nicotine dependence.

Intervention	Therapeutic Target	Phase	Study title	ClinicalTrials.gov Identifier
Fish oil	Modulation of dopaminergic neurotransmission	Phase 3	Studying the Effects of Administration of Polyunsaturated Fatty Acids (PUFAS) of Omega-3 Series in Nicotine dependence	NCT01735279
RS1051730	Nicotinic acetylcholine receptor gene	Not provided	Smokers Response to nicotine dependence Genotyping	NCT01780038
EVP-6124	Nicotinic Acetylcholine Receptor agonist	Phase 2	A Safety and Cognitive Function Study of EVP-6124 Versus Placebo in Subjects With nicotine dependence	NCT01480232
Nicotine Patch	Nicotine acetylcholine receptor Agonist	Phase 4	Nicotine Patch for Nicotine Dependence in Individuals With Schizophrenia or Schizoaffective Disorder - 1	NCT00046813
Nicoderm CQ transdermal Nicotine	Nicotine acetylcholine receptor	Phase 2	Assessment of High Dose Transdermal nicotine for Fast Metabolizers of Nicotine	NCT00956943
Varenicline	Nicotinic receptor partial agonist	Phase 3	Pharmacogenetics of Nicotine Addiction Treatment	NCT01314001
Bupropion	Dopaminergic receptor inhibitor	Phase 4	Bupropion for ADHD in Adolescents With Substance Use Disorder	NCT00936299
Naltrexone	Opioid receptor antagonist	Phase 2	Targeted Interventions for Weight-Concerned Smokers	NCT00105482
Chantix (Varenicline)	Nicotinic receptor partial agonist	Phase 2	Combination Bupropion / Varenicline for Smoking Cessation in Male Smoker	NCT01806779
Fluoxetine	Selective serotonin reuptake inhibitor **(SSRI)**	Phase 3	Fluoxetine as a Quit Smoking Aid for Depression-Prone Smokers	NCT00018174
Baclofen	Agonist for the GABAB receptors	Phase 2	Baclofen Effects in Cigarette Smokers	NCT01821560
Zyban	Dopamine reuptake inhibitor	Phase 1	Brain Reaction to Treatment of Nicotine Dependence	NCT00108173
Gemfibrozil	Activator of Peroxisome proliferator-activated receptor-alpha **(PPARα)**	Phase 2	Gemfibrozil for Nicotine Dependence	NCT01876810
Pioglitazone	Nuclear receptor peroxisome proliferator-activated receptor gamma **(PPAR-γ)**	Phase 1 Phase 2	Pioglitazone for Heroin and for Nicotine Dependence	NCT01395797
Selegiline	Selective irreversible MAO-B inhibitor	Phase 2	Selegiline Patch for Treatment of Nicotine Dependence	NCT01330030
Propranolol	Non-selective beta blocker	Phase 3	Memory Reconsolidation Blockade as a Novel Intervention for Nicotine Dependence	NCT00916721
Ondansetron	5-HT3 receptor antagonists	Phase 2	Role of Metabolites in Nicotine Dependence (3) – 6	NCT00000289
Cotinine fumarate	Desensitizes neuronal nicotinic acetylcholine receptors	Phase 2	Role of Metabolites in Nicotine Dependence (1) – 1	NCT00000284
GSK598809	Selective D3 Antagonist	Phase 2	Effectiveness of GSK598809, a Selective D3 Antagonist	NCT01188967
GW468816	NMDA Glycine Site Antagonist	Phase 2	Effectiveness of GW468816, an NMDA Glycine Site Antagonist, for Prevention of Relapse to Smoking	NCT00218465

Novel therapeutic targets/drugs being explored in nicotine dependence in randomized, double blind, placebo-controlled clinical trials and their associated interventions [56].

NEUROCHEMISTRY OF NICOTINIC RECEPTORS INVOLVED IN NICOTINE DEPENDENCE INDUCE WITHDRAWAL SYNDROME

Nicotine influences neuronal activity, synaptic communication, and finally behavior, through its effects on neuronal nicotinic receptors. These receptors have two or more agonist binding sites, which results in conformational change that leads to ion flux through the pore, inducing a depolarization and increased excitability [41]. The nAChRs are allosterically regulated, ligand-

gated ion channels consisting of five membrane spanning subunits [42]. nAChRs exist in 12 isoforms labeled α2 to α10 and β2 to β4. Each nAChR consist of five subunit molecules arranged in a ring around the central channel that opens to release ions when receptor gets activated [43]. The nAChR subtypes in mammalian brain are those containing α4 and β2 subunits [42]. In heteromeric nAChR have mix subunits for example two α4 and three β2 subunits. Mix subunits give receptors its distinct pharmacological properties, including response to nicotine stimulus [43]. nAChRs move to and fro between four dominant states: a). The resting state i.e polarized state b). The active state i.e depolarized state c). The desensitized state d). The inactive state (I: a long-lasting desensitised state) [3].

Resting of nAChRs: nAChRs receptors are in resting state and non- functioning, before binding to agonist [44]. Opening of nAChRs on binding with an agonist like nicotine transiently alter the conformation of the ion channel. The open channel permeates mainly sodium and potassium ions, depending on the nAChR subtype [45]. Closing and desensitization of nAChRs after opening for milliseconds, channel closes to resting state [44-45]. Nicotine acetylcholine receptors are integral membrane protein and prototypic members of ligand gated ion channels super family. They are formed by joining of 5 trans membrane subunits which are made up of 17 homologous polypeptide (alpha1-10, beta1-4, gamma, delta, and epsilon) in central nervous system, while, in the periphery, they mediate synaptic transmission at the neuromuscular junction and ganglia. nAChRs are also found in non-neuronal/nonmuscle cells [46]. nAChRs family is divided into two groups the acetylcholine muscle receptors, localized at the skeletal neuromuscular junction where they mediate release of various neuromuscular and their transmission, and the neuronal receptors, which are distributed throughout both the peripheral and central nervous systems. Muscle-type nAChR are pentameric complexes with four subunits; alpha, beta, delta and gamma in fetal receptors in the adult forms α, β, γ and € with two α1subunits in each pentamer [47]. Nicotinic acetylcholine receptors are present presynaptically and postsynaptically on nerve fibres. Presynaptically, these receptors help to regulate the release of neurotransmitters. Postsynaptically, the receptors facilitate fast synaptic transmission of impulse. Nicotine has a stronger interaction with the neuronal receptor. Thus, nicotine has the ability to rapidly cross the blood brain barrier [34].

The ligand **(ACh)** binding sites of the receptors consist of two parts one is principal and second one is complementary components. In case of homo pentameric (i.e. α7) receptors the same subunit carries on its opposite sides, both the principal and the complementary components so at the interface between two identical subunits the complete ligand-binding site comes into existence. Hetero pentameric receptor carries α (α2, α3, α4 or α6) subunits and β subunits (β2 or β4) as the complementary components of ligand-binding sites [48]. Dopamine areas arise from pedunculo pontine tegmentum **(PPT)** and latero dorsal tegmentum **(LDT)** which has collections of cholinergic neurons interspersed with GABAergic and glutamatergic neurons. The main neuronal projection of PPT is toward substantia nigra compacta and LDT project to VTA [49]. LDT and PPT both are responsible for drug seeking behavior [50]. Studies have shown that lesions in PPT

area reduces nicotine self-administration. VTA gets strong excitatory glutamate projections from prefrontal cortex that intervened on dopaminergic neurons that project revert to the cortex. It is found that PPT and LDT provide direct glutamatergic projections to dopamine neurons projecting nucleus Accumbens plays a major role in nicotine dependence induced withdrawal syndrome [45].

Table 2: Various nicotine receptors subunits and their role in nicotine dependence.

S. No.	Nicotinic receptors subunits	Brain Location	Neurotransmitter release	Type of Withdrawal syndrome
1.	α_7, β_2	Limbic System	Dopamine	Somatic withdrawal symptoms
2.	α_2, α_4, α_5, β_4	Medial habenula	Dopamine	Somatic withdrawal symptoms
3.	α_6	LC	Noradrenaline	Affective withdrawal symptoms
4.	β_2, α_7	Basal Ganglia	Dopamine	Somatic withdrawal symptoms
5.	α_2, α_5, β2,	IPN	Dopamine, Noradrenaline	Somatic withdrawal symptoms
6.	α_2, β_4	**VTA**	**Dopamine**	Somatic withdrawal symptoms

Various nicotine receptors subunits involved in somatic and affective withdrawal syndrome and their respective location in Brain.

β2 nAChRs

Essential for the ability of nicotine to depolarize DA cell bodies in the VTA and to increase their firing rate [42]. The behavioral effect of nicotine which is its self-administration is mediated by this nicotine receptor subunit [3]. Previous studies have shown that β2 nAChRs have role in social interaction, exploration strategies, decision making and rewarding effect of nicotine dependence [12].

β2 subunit

Gene linked with β subunit eliminates the behavioral effects of nicotine; reinserting the gene into the VTA restores behavioral responses to nicotine [16].

α7 nAChRs

Nicotine can potentiate glutamate input to DA neurons through α7 nAChRs [42]. These nAChR subunits are responsible for schizophrenia, due to activation of nAChRs, through glutamatergic excitation [51]. The α7 subtype also mediates cardiovascular effect of nicotine; it is thought to be involved in rapid synaptic transmission and may play an important role in learning and sensory gating [3] These subunits have highest calcium permeability. α7 displays voltage-sensitive blockade of the ion channel by Mg^{2+} and intracellular polyamines, so that they do not conduct current when the membrane is depolarized [47].

α7 and α4β2 subunits

nAChRs have allosteric-binding sites for their positive and negative modulators [48]. These subunits are responsible for modulation of glutamate release [52].

α4/β2 nAChRs

Activation of these subunits leads to depolarization of DA neurons in the VTA [42]. α4β2 containing nAChRs exhibited a negative affective or depression-like state, during electrical stimulition of their reward system but showed no somatic signs of nicotine withdrawal [43]. Pleasant effects of nicotine are due to β subunit receptors. Craving of nicotine symptom occur by desensitization of these beta receptors. Benowitz [3] showed a dose-dependent loss of Ca^{2+} entry when exposed to 0.1–10 mM nicotine for an hour or longer when exposed to embryonic kidney cells [3,44].

α4:β2 subunit

This subunit expressed in the ratio of 2:3, exhibited up-regulation after chronic nicotine exposure. Collectively, that data revealed the critical influence of subunit ratio in the nicotine-induced desensitization and upregulation of $\alpha4\beta_2$ subunit of nAChRs [47]. It is present in midbrain dopaminergic neurons. These receptors formed a high affinity binding complexes that are pentameric, trafficked to the cell surface, and produced acetylcholine mediated impulse [53]. $\alpha_4\beta_2$ receptor subunit is a heteromeric as well as pentameric membrane protein formed by two α and three β_2 subunits and is the most common nAChR subtype found within the brain. It has two agonist binding sites that have high affinity for nicotine and regulates release of the neurotransmitter dopamine through mesolimbic dopamine neurons [33]. α_7 and $\alpha_4\beta_2$ nAChRs have allosteric-binding sites for their positive and negative modulators [48].

α6 nAChRs

These subunits form pentameric receptors [53] and are responsible for nicotine self-administration [42]. It is found in less than 25% of GABA neurons [10]. This subunit in dopaminergic pathways contributes to spontaneous locomotor behavior, rewarding effect and attentional control in nicotine dependent rodents [4].

β4 nAChRs

This subunit in proopiomelanocortin **(POMC)** neurons in the arcuate nucleus of the hypothalamus is necessary for the appetite-suppressing effects and plays a prominent role in withdrawal symptoms of nicotine dependence [42].

α5/β2 nAChRs

α_5/β_2 subunit of nAChRs present on cortical glutamatergic projection neurons to the thalamus, are essential in maturation of this circuit and for normal adult performance in passive avoidance, a somatosensory aversive learning task in rodents [42].

α5, α5β4, α4β2 nAChRs

Stimulation of α_5 and $\alpha_5\beta_4$ nAChRs is important for the anxiogenic effects of nicotine [42]. α5 is found on GABA neurons [10]. α_5 increases sensitivity to nicotine activation of $\alpha_3\beta_2$ subunit. α_5

and β_3 subunits are unique in this classification because they carry neither the principal nor the complementary component of the ACh-binding site. This subunit is responsible for withdrawal symptoms of nicotine dependence [2].

α2 nAChRs

α2 nAChRs subunits produce a threonine-to-isoleucine replacement often associated with nicotine dependence [10].

α3 nAChRs

It is present in midbrain dopaminergic neurons circuits involved in nicotine reward and withdrawal [53].

α3β4 nAChRs

It mediates the cardiovascular effects of nicotine and also play a prominent role in nicotine aversion and withdrawal [3].

α5 nAChRs

These subunits of nAChRs are responsible for rewarding effect of nicotine dependence [4]. α5 subunit combined with α4β2, increases calcium conductance during nicotine exposure and has been associated with vulnerability to tobacco dependence in human. Activation of α5 gene variants also alter nicotine responsiveness in cultured human cells [16].

β3 nAChR

These subunits, in which the tyrosine residues of the complementary binding site loop E are replaced by phenylalanine, do not participate in agonist binding [54]. β3 subunits can influence levels of expression and agonist sensitivities of several nAChR subtypes towards nicotine. Consequently, they are considered accessory subunits [48]. β3 subunits located in striatum alter motor activity by means of modulating DA release during nicotine dependence [52].

α4 nAChR

nAChR on VTA is mainly composed of α4 sub unit [2]. Studies by [55] showed that these subunits contribute to spontaneous locomotor behavior and rewarding effect in nicotine dependent rodents. These subunits are present in ascending dopaminergic pathways [4]. This subunit is required for the transition from tonic to phasic firing that is crucial for reinforcement [4].

α6β2

It forms high affinity epibatidine binding complexes that are pentameric, trafficked to the cell surface, and produced acetylcholine evoked currents. α6β2 receptor up-regulation required higher nicotine concentrations than for α4β2 but lower than for α3β2 receptors. The α6β2 up-regulation occurred faster than for α4β2 and slightly earlier than for α3β2 during chronic nicotine exposure [53].

α3β2

This subunit is formed by high affinity epibatidine binding complexes that are pentameric, trafficked to the cell surface and produced acetylcholine evoked currents during acute nicotine exposure [53].

β2 subunits

These subunits control the GABA release and responses to ACh by DA neurons in mesencephalon. Activation of β2 nAChRs subunit facilitates nicotine self-administration [52].

APPLICATIONS TO OTHER ADDICTIONS AND SUBSTANCE MISUSE

Many drugs used for other indications e.g. anxiety, depression, alcoholism, morphine addiction, parkinson's disease and epilepsy, might be used to treat patients of nicotine addiction who are unable to quit smoking using standard, USA FDA approved, pharmacological interventions for nicotine dependence. Furthermore, several medications act on novel pharmacological targets like selective DA, epinephrine, glutamate, nACh and GABA receptors, as these play a prominent role in other addictions also like opium, alcohol, heroine, etc. These novel strategies often offer substantial promise to headstrong smokers for achieving cessation. This could lead to a significant reduction in the most deceptive, but preventable, substance-dependent disease, a common cause of death in humans. Emerging research suggests that simultaneous treatment of nicotine addiction and other addictions enhances the likelihood of success for both.

MINI DICTIONARY OF TERMS

Nicotine dependence

It is an addiction to nicotine. Nicotine is a psycho addictive drug which causes mood alteration changes in the brain which are pleasing, making people want to use again and again.

Nicotine acetylcholine receptors

Nicotinic acetylcholine receptors are ligand-gated ion channels comprising five membrane-straddling subunits that combine to form a functional receptor.

Limbic System

Limbic system is a group of structures which govern emotions and behavior It lies on both sides of the thalamus and cerebrum. The limbic system controls epinephrine release, emotion, behavior, motivation, memory and sense of smell.

Basal Ganglia

The basal ganglia are a collection of nuclei found on both sides of the thalamus and interconnected with the cerebral cortex, thalamus, and brainstem. Basic functions of basal ganglia are control of voluntary movements, cognition, learning, eye movements, and emotion.

Dopamine

It is a neurotransmitter of the catecholamine and phenethylamine families that controls the function of the human brain and body. It is an amine derived from L-DOPA. It plays a prominent role in nicotine addiction.

KEY FACTS REGARDING PHARMACOTHERAPY AND NEUROCHEMISTRY OF NICOTINE ADDICTION

- Tobacco consumption is a global disease that poses a substantial and costly health problem. There are some treatments options available, but they lack high levels of efficacy, particularly in real-life settings.
- Presently pharmacotherapy for the treatment of tobacco dependence is categorized into first line [nicotine replacement therapy **(NRT)**, bupropion and varenicline] and second-line medications (including nortriptyline and clonidine), according to clinical practice guidelines. Although these medications can also be used in combination.
- For the treatment of nicotine dependence several pharmacological agents that target acetylcholine, dopamine, glutamate, GABA, and endocannabinoid signaling systems have been anticipated and studied for their potential use.
- The release of extracellular dopamine **(DA)** in the nucleus accumbens **(NAcc)** is modulated by binding of nicotine to α4β2 and a7 nicotinic acetylcholine receptors **(nAChRs)** located on dopaminergic, glutamatergic and GABAergic neurons in the ventral tegmental area **(VTA)** of the midbrain.
- The rewarding and addictive effect of nicotine is due to release of DA in the NAc.

SUMMARY POINTS

- Tobacco smoking remains the leading preventable cause of death and illness in the world.
- The above chapter explains the neurochemistry based neurochemical mechanism by demonstrating a various pathway to understand mechanisms involved in nicotine dependence induced withdrawal syndrome through detailed actions of various nicotinic receptors and their subunit involvement on brain regions.
- Various pharmacological and non-pharmacological therapies have been used to prevent nicotine addiction and reduce the chances of reinforcement and relapsing conditions related to nicotine dependence.

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