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Perspective

Double target concept for smoking cessation

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Tobacco use is estimated to be the largest single cause of premature death in the world. Nicotine is the major addictive substance in tobacco products. After cigarette smoking, nicotine quickly acts on its target, nicotinic acetylcholine receptors (nAChRs), which are widely distributed throughout the mammalian central nervous system and are expressed as diverse subtypes on cell bodies, dendrites and/or nerve terminals. Through the nAChRs in brain reward circuits, nicotine alters dopaminergic (DA) neuronal function in the ventral tegmental area (VTA) and increases dopamine release from VTA to nuclear accumbens (NA), which leads to nicotine reward, tolerance and dependence. After quitting smoking, smokers experience withdrawal symptoms, including depression, irritability, difficulty concentrating or sleeping, headache, and tiredness. Recently, evidence has been accumulated to reveal the molecular and cellular mechanisms of nicotine reward, tolerance and dependence. The outcomes of these investigations provide pharmacological basis for smoking cessation. Here, I briefly summarize recent advancements of our understanding of nicotine reward, tolerance and dependence. Based on these understandings, I propose a double target hypothesis, in which nAChRs and dopamine release process are two important targets for smoking cessation. Dysfunction of nAChRs (antagonism or desensitization) is crucial to abolish nicotine dependence and the maintenance of an appropriate level of extracellular dopamine eliminates nicotine withdrawal syndromes. Therefore, the medications simultaneously act on these two targets should have the desired effect for smoking cessation. I discuss how to use this double target concept to interpret recent therapies and to develop new candidate compounds for smoking cessation.

Keywords: nicotine; nicotine addiction; nicotine reward; nicotine dependence; nicotine withdrawal syndromes; nicotinic acetylcholine receptor; smoking cessation

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Nicotine is a potent addictive substance in the tobacco that is thought to promote the use of tobacco products by about one-quarter of the world's population. Tobacco use is the leading preventable cause of disease, disability, and death. Cigarette smoking results in more than 400 000 premature deaths each year — about 1 in every 5 US deaths. Economically, more than $75 billion per year of total US healthcare costs is attributable directly to smoking. China is the world's largest producer and consumer of tobacco. It estimates that there are 0.35 billion cigarette smokers in China. Economically, more than $166 billion per year of total Chinese healthcare costs are attributable directly to smoking-associated diseases. Therefore, there is a considerable need to reduce the population of smokers. Unfortunately, nicotine addiction severely confounds attempts to end tobacco product use.

Nicotine addiction has been clinically delineated into two specific diagnosable disorders: dependence and withdrawal symptoms. Nicotine dependence refers to the maladaptive and chronic use of tobacco that meets the same types of criteria that are applied to other forms of drug addiction. Recent research has revealed two important features of nicotine addiction: (1) nAChRs play a critical role in developing nicotine addiction , and (2) nicotine addiction is a dynamic process including different stages such as nicotine-induced reward, tolerance, dependence and withdrawal-relapse symptoms[1]. Nicotine reward means that nicotine, acting on brain nAChRs, stimulates brain reward-associated circuits, which allows the smoker to be in a euphoric state. Dopamine is one of the key neurotransmitters actively involved within the reward circuits in the brain. Accumulating lines of evidence indicate that nicotine increases DA release from VTA to NA, which represents its nature of reward and intense addictive qualities.

Mounting evidence demonstrates that nAChR subtypes with different distributions within reward circuits mediate nicotine reward. Dopaminergic (DA) neurons in the VTA express diverse nAChR subunits, including α3–α7 and β2–β4[2–7], which can combine to form at least two pharmacologically distinct nAChR subtypes. One of these functionally

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distinct nAChRs is homomeric α7-nAChRs, which are mainly expressed on glutamatergic presynaptic terminals, where they mediate nicotine-induced increase of glutamate release onto DA neurons[6]. Another group of nAChRs is non-α7-nAChRs, which seems to be more complex. For example, electrophysiological studies, combined with single-cell RT-PCR technique, show $\alpha4\alpha6\alpha5(\beta2)_2$ or $\alpha4\alpha5(\beta2)_2$ combinations of nAChRs in midbrain reward center[6]. Immunoprecipitation approaches using nAChR subunit knockout mice show that functional α6β2-nAChRs are mainly located on DA neuronal terminals, whereas α4β2-nAChRs represent the majority of functional heteromeric nAChRs on DA neuron somata[8]. Interestingly, GABAergic neurons located in the VTA likely express relatively-simple nAChR subtypes, mainly $(\alpha4)_2(\beta2)_3$-nAChRs[6] and these α4β2nAChRs contribute to cholinergic modulation of GABA tonic release onto DA neurons[9]. Recently, we have characterized three functional subtypes (ID, IID, IIID) of nAChRs in VTA DA neurons[7] that may mediate nicotine reward and dependence. For example, nicotine, in the same concentrations and time ranges as obtained from cigarette smoking, enhances glutamatergic excitation in the VTA DA neurons by increasing glutamate release via stimulating presynaptic α7-nAChRs[10]. Nicotine also activates then desensitizes α4β2-nAChRs on both DA and GABAergic neurons[9]. Since cholinergic innervations in GABAergic neurons have much higher density than that in DA neurons [11], the nicotine-induced α4β2-nAChR desensitization (after brief activation) mainly decreases GABA release onto DA neurons, and consequently increases DA neuronal activity. It has been proposed that nicotine-induced desensitization is at least one of the major mechanisms for nicotine addiction[12–14]. In the VTA, nicotine activates (α7-) and/or desensitizes (α4β2) nAChRs, and in turn increases DA neuronal activity. The short-lived direct excitation of the DA neurons coupled with the enhanced glutamatergic afferent activity provides the presynaptic and postsynaptic coincidence necessary to initiate synaptic potentiation and plasticity (LTP)[15, 16]. Taken together, these synaptic events lead to a relatively long-lasting heightened activity of midbrain DA neurons, which release more DA to NA and results in positive reinforcement. The nicotine reward is an important early event to initiate and develop to nicotine dependence.

As mention before, nicotine, in addition to activating nAChRs, also desensitizes them [17], which plays an important role in the developing nicotine tolerance and dependence. After repetitive exposure to nicotine, nAChR desensitization represents a low functional status of nAChRs in the presence of low level of nicotine, and the recovery from receptor desensitization is relative slow. Under this desensitization condition, the same concentrations of nicotine are not enough to induce the same level of reward signals (the level of extracellular DA in the NA), which consequently causes nicotine tolerance. On one hand, to overcome nicotine tolerance (to get the same level of reward stimulation), smokers increase cigarette smoked per day, which results in the nicotine dependence. On the other hand, nAChR desensitization triggers receptor upregulation, and the upregulated nAChRs imbalances cholinergic signaling and modulation, which causes withdrawal symptoms when smoker quits cigarette smoking.

In summary, nicotine addiction is the major reason why smokers continue to rely on tobacco products. Nicotine dependence and withdrawal symptoms are two pathophysiological changes brought on by cigarette smoking. Based on these lines of evidence, I propose a "double target" hypothesis to interpret pharmacotherapeutic mechanisms of smoking cessation. In this hypothesis, I assume that the medications that eliminate both nicotine dependence and withdrawal-relapse will exert more desired effect for smoking cessation (Figure 1). To reduce nicotine dependence, medications should block nAChRs (in particular α4β2-nAChRs), while to eliminate withdrawal-relapse, they also should appropriately (mildly) increase extracellular DA level in the NA.

Using this double target concept, one can explain pharmacological mechanisms of existing smoking cessation medications. For instance, recent market drugs available for smoking cessation are bupropion and varenicline[18]. Bupropion is used to treat mental depression, but it is also used as part of a support program to help people stop smoking[19]. This medicine exhibits double target properties since it not only blocks dopamine transporter (maintain a mildly elevated level of extracellular DA in the NA), but also blocks nAChRs, in particular α4β2-nAChRs[19–21]. Varenicline is another example of a smoking

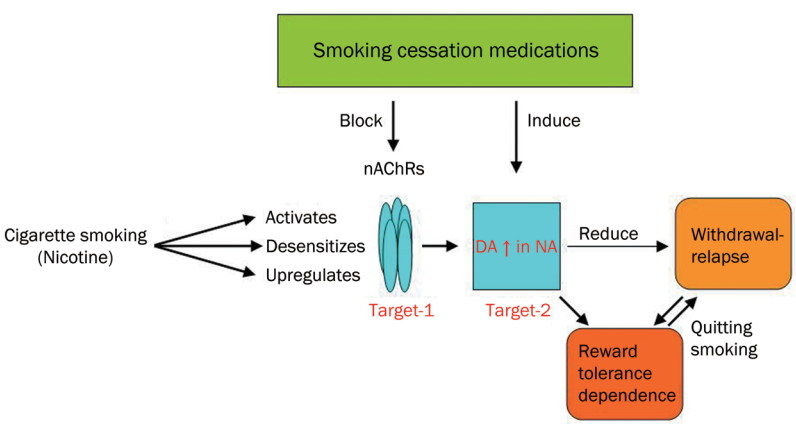

Figure 1. Scheme of double target hypothesis for smoking cessation. Target 1 is the nAChRs that is critical for nicotine reward, tolerance and dependence, while target 2 is the maintenance of mild extracellular DA level in the rewarding circuit (NA), which eliminates withdrawal-relapse syndromes.

cessation medication[22] that matches the double target concept. It is a partial agonist at α4β2-nAChRs, and is also a full agonist at α7 nAChRs[23]. As a partial agonist of α4β2-nAChRs, varenicline mimics nicotinic effect to activate/desensitize its first target, α4β2-nAChR, and diminishes nicotine dependence. In addition, as a full agonist at α7 nAChRs, it also mimics nicotinic stimulation on its second target, presynaptic α7-nAChR, to increase glutamate release onto DA neurons in the VTA, which in turn appropriately increases DA release from the VTA to NA, and eliminates withdrawal symptoms.

Based on this double target concept, one also can develop new medications for smoking cessation. Recently, my laboratory has developed two different compounds that exhibit high potential to be novel smoking cessation medications. One group of compounds is called tetrahydroprotoberberine analogs (THPBs) which include tetrahydroberberine (THB), l-stepholidine (l-SPD) and l-hydroparmatine (l-THP). THPBs are purified from several Chinese herbs in the magnoliidae superorder. Mounting evidence indicates that THPBs exhibit dopamine receptor (D_2) antagonist effects on sedation, hypnosis, antinociception, anti-schizophrenia and anti-hypertension[24–27]. The major pharmacological targets for THPBs are dopamine receptors, and THPBs exhibit D1 partial agonist and D2 antagonist effects. As a D2 receptor antagonist in VTA DA neurons, systemic exposure to THPBs increase DA neuronal firing by blocking D2 auto-receptors on DA neurons and increase DA level in NA. It has been reported that l-THP effectively eliminates heroin addiction in human[28], suggesting that THPBs may have a potential for treating drug addiction. Recent research in my laboratory also shows that THPBs potently inhibit either human α4β2-nAChRs heterologously expressed in SH-EP1 cell line or rodent α4β2-nAChRs in midbrain DA neurons. This double-target feature suggests that THPBs are good candidates that can be developed into new smoking cessation medications.

Another compound we are working on is iptakalim hydrochloride (Ipt). Ipt was initially designed and synthesized as an antihypertensive drug[29]. It is a small, water soluble molecule that freely penetrates the blood-brain barrier and has minimal toxic side effects following long-term systemic administration[30]. Possible pharmacological mechanisms underlying its antihypertensive action include K_{ATP} channel activation and endothelin antagonism. Tests in a variety of *in vivo* and *in vitro* ischemia and Parkinson's disease models indicate that Ipt has neuroprotective effects[30–32]. Furthermore, Ipt can potentially prevent drug addiction since it inhibits cocaine challenge-induced enhancement of dopamine release in rat NA[33]. The major pharmacological target of Ipi is thought to the cytoplasmic and/or mitochondrial K_{ATP} channels[30]. My laboratory has evaluated the effects of Ipt on nAChR function and found that Ipt potently blocks α4β2-nAChRs heterologously expressed in human SH-EP1 cell line[34] or natively expressed in rat midbrain DA neurons[35]. Currently, we have also found that Ipt significantly prevents systemic nicotine-induced behavioral (locomotor activity) sensitization, further confirmed its nAChR antagonism (Wu *et al*, unpublished data). Interestingly, systemic injections of Ipt along (30 $mg \cdot kg^{-1} \cdot day^{-1}$, ip for 7 days) increase rat locomotor activity (Wu *et al*, unpublished data), suggesting that on one hand, Ipt can block α4β2-nAChR function, and on the other hand, it also can appropriately increase DA level at NA although the reason and mechanisms of this effect are still unclear. Nevertheless, Ipt also exhibits double-target feature and it likely can be developed as a novel smoking cessation medication.

In conclusion, nicotine addiction is a complex brain disorder, which involves functional alterations in multiple brain circuits and exhibits different stages. Based on the new concept of network pharmacology[36, 37], I propose a double target hypothesis, which may help to understand the pharmacotherapeutic mechanisms of smoking cessation, and also help to develop new medications for quitting tobacco use.

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