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Tobacco addiction: A biochemical model of nicotine dependence

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SUMMARY

Nicotine is the main psychoactive substance present in tobacco, targeting in the CNS the nicotinic acetylcholine receptors (nAChR). The main effects of nicotine associated with smoking are nAChR upregulation, nAChR desensitization and modulation of the dopaminergic system. However, there is a lack of a comprehensive explanation of their roles that effectively makes clear how nicotine dependence might be established on those grounds. Receptor upregulation is an unusual effect for a drug of abuse, because theoretically this implies less need for drug consumption. Receptor upregulation and receptor desensitization are commonly viewed as opposite, homeostatic mechanisms. We here analyze the available information under a model in which both receptor upregulation and receptor desensitization are responsible for establishing a mechanism of nicotine dependence, consequently having an important role in starting and maintaining tobacco addiction. We propose that negative feedbacks on dopamine release regulated by $\alpha4\beta2$ nAChRs are disrupted by nicotine. nAChR desensitization is the disrupting mechanism, while nAChR upregulation is the reinforcing process of nicotine dependence, which eventually initiates tobacco addiction. A conclusion of the model is that drugs used for smoking cessation should inhibit preferentially $\alpha4\beta2$ nAChRs and to have a low or null ability to upregulate nAChRs, as this characteristic allows the smoker to achieve downregulation without abstinence symptoms. A relationship between this hypothesis and smoking and schizophrenia is also discussed.

Introduction

According to the World Health Organization, tobacco addiction is a global health problem affecting one-third of the population. Around half of the smoking population dies from a smoking associated disease. The evidence points to the alkaloid nicotine as the principal psychoactive substance present in tobacco [1].

A full understanding of the mechanisms by which nicotine promotes tobacco addiction is lacking. Nicotine is an agonist of nicotinic acetylcholine receptors (nAChRs), which are important neuronal modulatory proteins that also mediate muscle activation in vertebrates. They are pentameric ion channels belonging to the large "cys-loop" superfamily of receptors [2]. With 12 different subunits ($\alpha2$–$\alpha10$, $\beta2$–$\beta4$), the receptor variability is potentially large [3].

Three main processes have been related to tobacco addiction: nAChR modulation of the dopaminergic and glutamatergic systems, nAChR upregulation by nicotine and nAChR desensitization by nicotine.

nAChR modulation of the dopaminergic and glutamatergic systems

Dopamine (DA) release modulation by nAChRs has been well documented and it is very important for the development of tobacco addiction [1,4,5]. The dopaminergic system is central to reinforcing behaviors, reward and the development of drug abuse and dependence; drugs of abuse increase in the nucleus accumbens (NAc) dopamine levels [6]. The mesocorticolimbic dopamine system comprises cell bodies from the ventral tegmental area (VTA) and their projections to the NAc. There is an increase of dopamine release in the NAc and activation of DA neurons in the VTA by nicotine concentrations that are obtained by smokers [5]. Systemic nicotine increases the activity of DA neurons in the VTA via N-methyl-D-aspartate (NMDA) glutamate receptors on DA neuron cell bodies [7]. This increase is mediated by $\alpha7$ receptors present on the glutamatergic terminals, facilitating glutamate release [8,9]. Additionally, nicotinic agonists improve cognition [10] inducing long-term potentiation (LTP) through a hippocampal glutamatergic system similar to the described for the VTA.

nAChR upregulation

The classical idea is that upregulation of nAChRs results from an increase in receptor number after a chronic exposure [11,12]. This view was challenged by the interpretation that the number of

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receptors remains unchanged, but the functional condition is altered to a "high-affinity state", easier to activate and with a larger single-channel conductance [13]. More recently, the classical idea was reinforced [14]. In this model nicotine promotes upregulation acting as a molecular chaperone stabilizing assembly intermediates, and by increasing the half-life of nAChRs [14,15]; the "high-affinity state" hypothesis was reinterpreted as an increase in the proportion of the $(\alpha4)_2(\beta2)_3$ stoichiometry.

Upregulation by nicotine, characteristic of α4β2 receptors, has been referred as a central mechanism somehow related to nicotine addiction [16]. α4β2 receptors are the most abundant nAChRs in the brain and are directly involved in tobacco addiction. They have high-affinity for nicotine [17] and high-affinity nicotine binding sites in most brain regions are formed from α4β2 nAChRs [18,19].

nAChR desensitization

It was proposed that desensitization, a reversible reduction in response due to chronic agonist exposure [20] triggers upregulation [21]. nAChRs can exist in three basic physiologic states: resting, activated and desensitized. In the resting state, the receptor channel is closed, the two agonist binding sites are empty and with low affinity for ligands. Upon ligand binding at high-agonist concentrations, the channel transiently opens. This activated state is followed by a desensitized or inactive conformation where the binding sites are occupied with high-affinity. After agonist removal, the receptor goes back to the resting state and is ready for a new activation cycle. However, low agonist concentrations can induce desensitization even without activation, a process named "high-affinity desensitization" [20]. This is a slow process that in the presence of nicotine affects preferentially α4β2 receptors compared to α7 [22]. At nicotine concentrations present in smokers, α4β2 are desensitized and upregulated but not α7 receptors [22].

Physiological roles of desensitization and upregulation in tobacco addiction

Because nicotine promotes both, upregulation and desensitization are thought to be central to nicotine addiction. Desensitization is an intrinsic feature of the molecular structure of nAChRs [20] and nicotine is highly effective in promoting it, especially in the α4β2 receptor. Upregulation on the other hand, has only been observed after external administration of agonists, especially nicotine. Upregulation by nicotine is at first view paradoxical [23], because the number of receptors is increased in a sort of positive feedback, contrary to what is normally expected for a drug of abuse. One possible explanation is that this increment is a homeostatic response to desensitization [16,24]. In contrast, others have proposed that desensitization might represent a cellular basis of nicotine tolerance [5], especially in the presence of upregulation.

The described explanations for upregulation (homeostatic response to desensitization) and desensitization (a mechanism for nicotine tolerance) make emphasis on their independent roles as putative corrective mechanisms, but they have no direct relationship with a mechanism of nicotine dependence. Furthermore, they are not compatible because only one can represent a problem that the brain must compensate. If nicotine activation of nAChRs is a problem and needs to be balanced by desensitization, then upregulation, going in the opposite direction, should not be needed. However, if desensitization is the main disruptive process, then upregulation does make sense as a way to compensate the lost signaling capability. However, none of these scenarios explains how nicotine dependence is initiated.

The problem is that nicotine triggers both desensitization and upregulation, and reasonably these mechanisms have been proposed to be involved in nicotine dependence. Recently the dual role of nicotine, activation and desensitization of nAChRs, was comprehensively described in the context of tobacco addiction [25]. They show that a coordinated activation and desensitization of different nAChRs on different neuronal subtypes occurs in response to nicotine administration during smoking.

Nicotine dependence and tobacco addiction

As with other drug addictions, a difference between the mechanisms underlying tobacco addiction and nicotine dependence exists [26]. The former has a broader connotation, including complex behavioral components. The latter could be restricted to a specific biochemical alteration triggered by nicotine, an alteration that would promote the physical need for more nicotine to compensate the disarrangement. Clearly, a behavioral component in tobacco addiction is essential. Subjects need to relate the experienced pleasure with its origin (i.e. nicotine self-administration devices and tobacco cigarettes). In other words, smokers are addicted to cigarettes not to nicotine, but only because cigarettes and the various associated behaviors result in nicotine administration.

The initial and intrinsic biochemical basis of tobacco addiction should be found in the abnormal mechanisms nicotine promotes on nAChRs (i.e. not promoted by ACh). These mechanisms, according to our proposal, are nAChR upregulation and desensitization. Based on the current literature, we propose a biochemical mechanism that can explain or give a molecular basis to the habit-forming properties of nicotine, likewise the inhibition of dopamine reuptake by cocaine explains a part of its dependence properties [27]. We describe a mechanism under which desensitization and upregulation are compatible molecular processes that effectively cause nicotine dependence. That is, they are not considered just opposing homeostatic processes.

Desensitization and upregulation as the basis for nicotine dependence

We involve in this analysis the biochemical processes associated to nicotine dependence, excluding behavioral components, restricting our reasoning to upregulation and desensitization.

Hypothesis I: nicotine activation of nAChRs as the basis of nicotine dependence

In this scenario nicotine exacerbates nAChR *activation*, and by increasing dopamine release, promotes all the rewarding mechanisms associated with addiction through the DA system. As we show later, there are well described neuronal circuits in which nAChR activation enhances DA release. However, nicotine also triggers desensitization which cancels what in principle we assumed promotes addiction. If we stop the reasoning here, then it would be logical that smokers need more nicotine to receive again the same rewarding stimuli (albeit they would need to wait until the receptors go from the desensitized to the resting state). However, nicotine also promotes upregulation, so in a short time the brain would develop an exacerbated capacity of nAChR response (positive feedback) when the receptors are not desensitized. If so, an immediate need for more nicotine would be lacking, as the normal concentration of the natural agonist ACh would be sufficient to elicit a response similar to the first nicotine stimulus. Moreover, without an immediate need for more nicotine, no reinforcing behavioral mechanisms are developed. Under this circumstances only when upregulation is reversed, nicotine consumption might be eventually wanted, but not needed in a dependence way. That is, this hypothesis does not explain addiction.

Hypothesis II: nicotine inactivation of nAChRs as the basis of nicotine dependence

As a second scenario, we assume that it is nAChR *inactivation* by nicotine desensitization, what initiates the dependence mechanism and eventually addiction. In this case nAChR inactivation enhances DA release, for example, through the desensitization of nAChRs present on inhibitory GABAergic neurons (see below). A logical consequence is that the upregulation that follows desensitization completes the mechanism of nicotine dependence. In effect, independently of the mechanisms triggered by nAChR desensitization to enhance DA release (see below), upregulation makes more difficult to achieve inactivation (and rewarding). Consequently, upregulation promotes the need of higher or faster nicotine consumption. Therefore, in this hypothesis nicotine provokes both the rewarding stimulus (via desensitization) and the need for more nicotine consumption (via upregulation).

Nicotine activation of rewarding: disrupting a negative feedback mediated by α4β2

We concluded that in order to conciliate desensitization and upregulation in a possible mechanism of nicotine dependence, nicotine has to inactivate nAChRs as proposed by hypothesis II, described in the previous section. Fig. 1 shows this process schematically and explicitly proposes that what desensitization inactivates is an inhibition mechanism of rewarding stimulus mediated by α4β2 nAChRs. Beginning with a basal number of nAChRs, endogenous ACh normally stimulates all types of receptors, and depending on the neuronal circuit, activates or inhibits rewarding stimulus. For this purpose, we distinguish three basic neuronal processes related to rewarding. The assumption is that the increase in dopamine release correlates with rewarding activation, while inhibition of dopamine release means rewarding inhibition. These processes are Long-Term Rewarding Activation (LTRA), Short-Term Rewarding Activation (STRA), and Long-Term Rewarding Inhibition (LTRI).

LTRA is associated with glutamatergic systems [7], as described above. Because ACh or nicotine activate an indirect complex system of dopamine release, the removal of these agonists does not cause an immediate stop of rewarding activation. Fig. 2 shows a diagrammatic representation of this and the other neuronal systems described in subsequent sections.

STRA (Fig. 2) is mediated by nAChRs present on cell bodies and terminals of DA neurons in the VTA. These nAChRs are of the α4β2, $(\alpha4)_2\alpha5(\beta2)_2$, $\alpha4\alpha6\alpha5(\beta2)_2$ and α7 types [28,29], and when activated by ACh or nicotine promote the liberation of dopamine. Dopamine release stops as soon as ACh or nicotine is removed or nAChRs are desensitized.

An example of LTRI is the important GABAergic system involved in the regulation of dopamine release in the VTA. This system inhibits DA neurons (Fig. 2), and is activated by α4β2 receptors [28,30].

If instead of the endogenous ACh ("normal path"), these systems receive an acute exposure to nicotine ("disrupted path"), the same results are obtained but transitorily (Fig. 1). Nicotine is not degraded as ACh and remains at low concentration for longer periods. After the normal recovery of nAChRs, and as expected for a chronic exposure, the residual low nicotine concentration desensitizes preferentially α4β2 nAChRs [22] involved in rewarding inhibition. This provides a long-lasting rewarding stimulus not present in the normal path. The ability of low nicotine concentrations to preferentially desensitize α4β2 compared to other receptors, allows LTRA and STRA to remain active. The rewarding effects end when the inhibited α4β2 nAChRs are upregulated and exit the desensitized state after nicotine removal [14]. The result

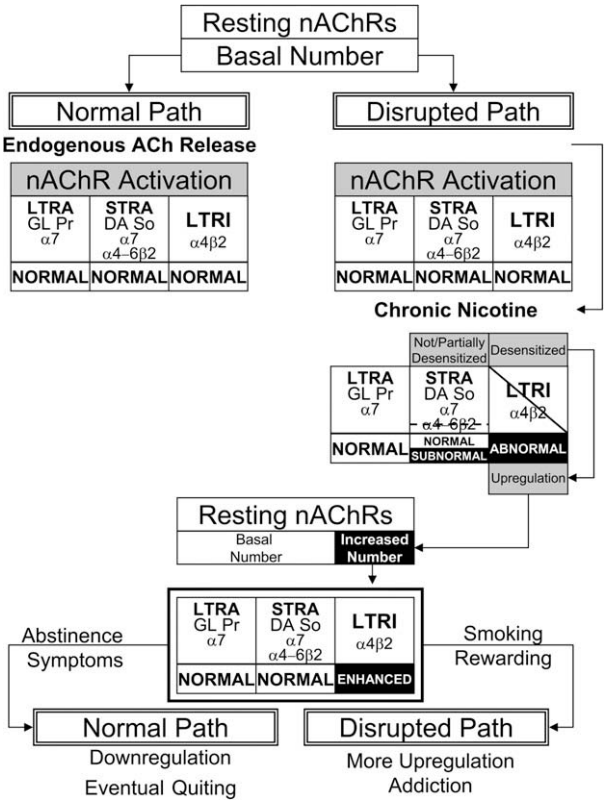

Fig. 1. Schematic representation of the mechanisms by which nicotine dependence is established. The model distinguishes three neuronal processes related to rewarding: LTRA, Long-Term Rewarding Activation; STRA, Short-Term Rewarding Activation; LTRI, Long-Term Rewarding Inhibition. The assumption is that an increase in dopamine release correlates with rewarding activation, while inhibition of dopamine release means rewarding inhibition. LTRA is associated with glutamatergic (GL) neuronal circuits, where nicotine increases the activity of dopaminergic (DA) neurons in the VTA. This activation is mediated by presynaptic (Pr) nAChR α7 receptors on glutamatergic neurons, which facilitate their excitation. The target NMDA glutamate receptors are present on the cell bodies of DA neurons. This rewarding path is considered long-lasting because it is indirectly activated by ACh or nicotine, and the removal of this agonist has no immediate consequences and α7 receptors are not desensitized by nicotine. STRA is mediated by somatic (So) nAChRs localized on DA neurons cell bodies in the VTA. The nAChRs types involved are less or not sensitive to nicotine desensitization (see text) and when activated enhance the release of dopamine. Dopamine release (and rewarding) stops as soon as ACh or nicotine is removed. The essential feature of LTRI is that is mediated by the α4β2 type of nAChR, characteristic for its high-propensity to desensitization by nicotine. A well documented example is the modulation of the GABAergic system. A basal number of all types of resting nAChRs are stimulated by endogenous ACh in a normal physiological path (left), and depending on the neuronal circuit and subunits involved, activates or inhibits rewarding stimulus. On the other hand, after an acute administration of nicotine, the normal functioning of the nicotinic cholinergic circuit is disrupted (right). Transitorily, nicotine has the same effects of the endogenous ACh. However, nicotine is not cleaved as ACh and consequently (solid arrows represent causal relationships) it remains for a longer period at low concentration (chronic state). Nicotine, at this low concentration, has different consequences in the three neuronal processes. As α7 is not desensitized by nicotine, the effect on LTRA is a prolonged activation. Because STRA is not based on the α4β2 type, it is either continuously activated, or only partially inhibited (dashed lines). LTRI, based on α4β2, is fully (solid line) inhibited by desensitization. The overall result at this stage is an exacerbation of the rewarding system by the three neuronal processes. However, desensitization of α4β2 in LTRI promotes their upregulation. Therefore, in the subsequent population of resting nAChRs, the α4β2 type increases its number (or changes its physiological state, see text) enhancing the inhibitory circuit. What follows depends on the smoker behavior. The easiest way to recover a normal rewarding level is to administer more nicotine (bottom right). However, this is a transient solution, as it also reinforces the disrupted path through a new cycle of α4β2 desensitization–upregulation, increasing rewarding inhibition. The alternative choice is to wait unpleasantly until α4β2 receptors downregulate to normal levels (bottom left).

is an exacerbated (upregulated) rewarding inhibition system under normal conditions. The smoker faces two alternatives at this stage.

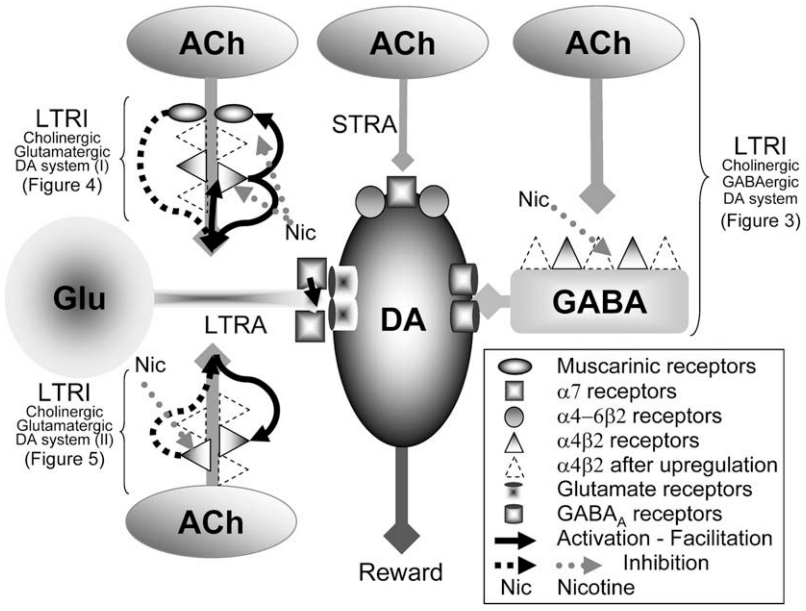

Fig. 2. Schematic representation of neuronal systems involved in nicotine addiction. This diagram shows how dopamine release (rewarding) is modulated by neuronal circuits involved in nicotine addiction. Actual receptor neuronal localization and their modulatory relationships are represented (positive and negative feedbacks). Neuronal circuits described in subsequent figures are indicated. LTRA: Long-Term Rewarding Activation; STRA: Short-Term Rewarding Activation; LTRI: Long-Term Rewarding Inhibition; ACh: cholinergic neuron; DA: dopaminergic neuron; GABA: gabaergic neuron; Glu: glutamatergic neuron. For other references see the inset.

Either to suffer the consequences of the abstinence symptoms until nAChRs are downregulated (interrupting the addiction process or eventually quitting smoking), or to consume more nicotine to disrupt by desensitization the exacerbated rewarding inhibitory system. The latter (easy) choice has the malicious consequence of a further and long-lasting α4β2 upregulation, favoring addiction. In short, desensitization and upregulation are not viewed as regulatory mechanisms, but as abnormal consequences of nicotine exposure.

Neuronal circuits compatible with the desensitization–upregulation hypothesis

In the following sections we propose actual examples of neuronal circuits matching the theoretical framework presented. All of them can coexist and therefore nicotine dependence can be initiated and reinforced simultaneously in different brain regions.

Cholinergic–GABAergic–DA system

An example of LTRI compatible with our proposal, the inhibitory GABAergic neuronal circuit, is shown in Figs. 2 and 3. The inhibitory mechanism here is the hyperpolarization of DA neurons by $GABA_A$ receptors. Although nicotine transiently enhances the inhibitory GABAergic transmission on DA neurons via α4β2 activation, a longer rewarding stimulus follows via α4β2 desensitization [30]. This desensitization promotes an enhanced release of DA in the NAc. nAChRs on DA neurons can be also transiently activated (STRA) and subsequently inhibited. However, the long-lasting effects of canceling LTRI prevail. First, many of nAChRs in DA neurons are of the α7, $\alpha4\alpha6\alpha5(\beta2)_2$ and $(\alpha4)_2\alpha5(\beta2)_2$ types [28,29]. This means a null or a reduced desensitization promoted by nicotine because the β2 subunit is either not present or present in less proportion in these receptors. This view is supported because nAChRs on GABAergic neurons are upregulated, but not those on dopaminergic neurons [31]. Second, the majority of the cholinergic neurons in the VTA project to GABAergic neurons [32]. Thus, the

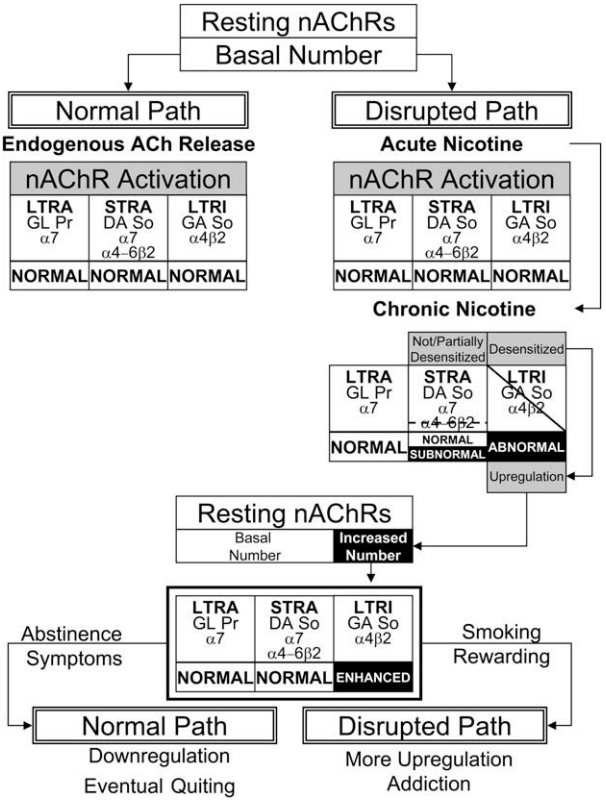

Fig. 3. Schematic representation of the mechanisms by which nicotine dependence is established by inhibition of a GABAergic neuronal circuit. The well documented inhibitory mechanism based on the GABAergic (GA) hyperpolarization of DA neurons is represented as an example of the general LTRI of Fig. 1. Although nicotine transiently enhances the inhibitory GABAergic transmission on DA neurons via α4β2 activation (for abbreviations see Fig. 1), a long-term rewarding stimulus follows through α4β2 desensitization. However, the initial transient inhibition become stronger (hyperpolarization is enhanced) with each cycle of upregulation.

eventual inactivation of nAChRs on DA neurons is probably physiologically less relevant.

During desensitization, α7 mediated LTRA remains capable of nicotine and endogenous ACh activation [30]. However, with time, upregulation of α4β2 receptors reinforces the inhibitory pathway. Thus, as a consequence of upregulation, a sustained and probably increased exposure to nicotine is needed not just to enhance but to maintain DA release within the normal levels. As we saw, the easier decision to alleviate the unpleasant symptoms is to smoke again, but subsequent exposures to nicotine reinforce this mechanism. In other words, a biochemical mechanism to start tobacco addiction is established.

Cholinergic–glutamatergic–DA system (I)

A second neuronal circuit compatible with our hypothesis is the glutamatergic path involved in LTRA (Figs. 2 and 4). Activation of α7 receptors by nicotine promotes dopaminergic activity via facilitation of glutamatergic pathways, but this fact alone does not explain nicotine dependence. Their upregulation (if any), which would eventually reinforce dopamine release, does not explain a need for more nicotine as described above for the GABAergic system.

While activation by acute doses is important transiently, the key point to develop addiction in our hypothesis is the assumption that the main effect of nicotine in nicotine dependence is inactivation of nAChRs via desensitization. The inactivated inhibitory mechanism in this case is a negative feedback on ACh release (LTRI), located in *cholinergic* fibers that activate α7 receptors on glutamatergic neurons (Fig. 2). α4β2 presynaptic ionotropic receptors have a positive feedback role on ACh release while metabotropic muscarinic receptors are involved in a negative feedback [33]. In the nervous system, there are several registered cases of positive feedback on ACh release [34–37]. However, the muscarinic negative feedback seems to be activated only after an overflow of ACh release and to be mediated by activation of α4β2 receptors [35]. As a metabotropic receptor, the effect of the muscarinic type is long-lasting. We propose that if α4β2 receptors are desensitized by nicotine, then the negative muscarinic feedback cannot start, but the activation of α7 is still enhanced. Effectively, the main result of the positive feedback mediated by α4β2 is an overflow of ACh. The lack of this overflow suppresses both, the negative feedback and a massive and simultaneous activation of postsynaptic α7. However, without a negative feedback, the activation of the cholinergic fibers is still capable of producing a longer and continuous (tonic) activation of the postsynaptic α7 receptors, which are not desensitized at low nicotine concentrations. Likewise the previous neuronal circuit, rewarding activation remains whilst rewarding inhibition is abolished.

In the GABAergic path, both endogenous ACh and acute nicotine exposure have the same effects (Fig. 3). Here, an acute exposure to nicotine does not *per se* enhance ACh release unless concomitant with the activation of the cholinergic fiber. Even if it does promote some ACh release by activating presynaptic α4β2 receptors, it most probably does not activate the negative feedback mediated by muscarinic receptors without the presence of an action potential.

The important fact is that α7 receptors, which promote dopamine release, are not desensitized and upregulated by nicotine. Fig. 4 shows that both effects of α4β2 are enhanced, the positive feedback on ACh release targeted to postsynaptic α7, and the negative feedback on ACh targeted to muscarinic receptors. However, the effect of the enhanced positive feedback on ACh release is not directly proportional to the level of α4β2 upregulation as it is limited to the *activation* of a "fixed" number of α7 (represented by a dashed line in Fig. 4). On the contrary, the enhanced negative feedback on ACh is directly proportional to the levels of presynaptic α4β2 (which are upregulated) and of ACh release, which is highly increased. Thus, the higher the concentration of ACh the faster the onset time of the negative feedback. In this system, with a large population of upregulated α4β2, the net effect of an action potential is a full but very short activation of postsynaptic α7 receptors,

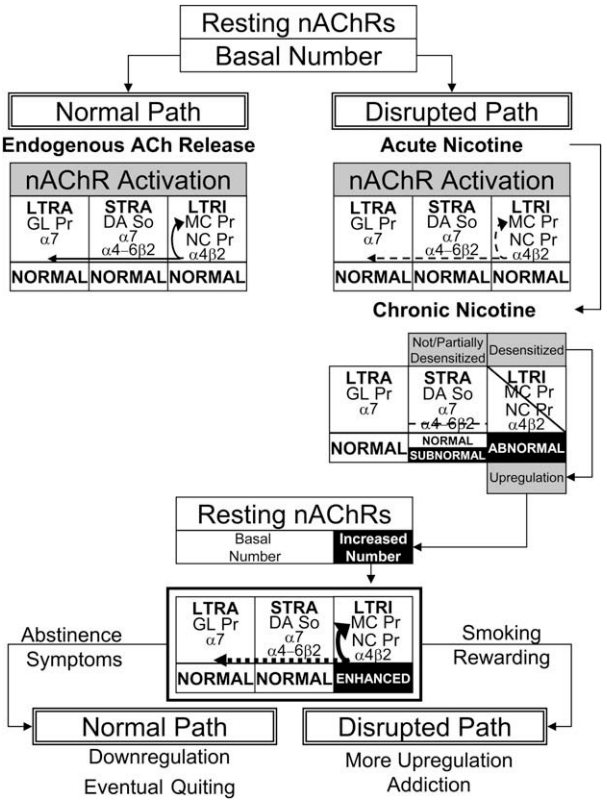

Fig. 4. Schematic representation of the mechanisms by which nicotine addiction is established by inhibition of a negative feedback that modulates a Glutamatergic neuronal circuit (Case I). The LTRI suppressed by nicotine in this instance is a negative feedback on ACh release based on α4β2 receptors. This ACh release is involved in the activation of postsynaptic α7 receptors situated on glutamatergic neurons, which in turn facilitates dopamine release. Presynaptic regulation of ACh release is usually positive by nicotinic (NC) α4β2 receptors, and negative by muscarinic (MC) receptors (for other abbreviations and further explanations see Fig. 1 and the text). Muscarinic inhibition of ACh release is triggered by both, an activation of α4β2 receptors and by an overflow of ACh (which in turn is also facilitated by the activation of α4β2 receptors). The arrows within the boxes show the dual effect of the presynaptic α4β2 receptors. On the one hand, they enhance ACh release and consequently they increase the simultaneous activation of α7 receptors on the glutamatergic postsynaptic side (indirect increase of dopamine release via facilitation of glutamatergic transmission). On the other hand, they allow a subsequent activation of the negative feedback mediated by the muscarinic receptors as a side effect of the increased ACh release (reduced activity of glutamatergic neurons, decrease of dopamine release). After an acute administration of nicotine, ACh release is highly enhanced if concomitant with an action potential, but also an early activation of the muscarinic inhibition is launched. Without an action potential, α4β2 receptors might induce the liberation of some ACh and activate α7 receptors on the glutamatergic neurons, without triggering muscarinic inhibition. As before, α7 are also directly activated by nicotine. Dashed lines indicate this conditional or reduced modulation. With chronic nicotine α4β2 receptors are desensitized, the positive feedback on ACh release and the overflow of this neurotransmitter are abolished, and consequently the negative feedback is not activated. The result of these events is a lower but continuous or prolonged activation of postsynaptic α7 receptors which facilitates glutamatergic and subsequently DA transmission. α4β2 upregulation promotes an enhanced modulation (bold arrows) that is asymmetrical in its consequences. Upregulated α4β2 receptors now trigger the muscarinic negative feedback faster, proportionally to their increased number and to the increased ACh overflow (see also the text). However, the enhanced activation of glutamatergic neurons is still limited by the number of postsynaptic α7, which are not upregulated.

and a very fast (and probably longer) activation of the muscarinic negative feedback of ACh release. As with the GABAergic system, the initial rewarding effect of nicotine ends with a long-lasting reduction in the activation of the DA system. This reduction of dopamine release eventually ends when α4β2 receptors are downregulated. However, as before smokers might reduce this time to avoid abstinence symptoms by means of nicotine consumption, neutralizing the upregulated α4β2 nAChRs via desensitization. This will in turn maintain in the long-term a great proportion of α4β2 nAChRs in the upregulated state perpetuating nicotine use.

In the NAc, excitatory postsynaptic currents (EPSCs) mediated by AMPA/kainate (KA) and NMDA receptors are inhibited when ACh or carbachol are added to the superfusing medium [38]. In contrast, in the presence of the muscarinic antagonist atropine, these neurotransmitters increase by 35% the AMPA/KA and NMDA receptor-mediated EPSCs. These excitatory effects were blocked by the nicotinic antagonist mecamylamine, which is more potent blocking α4β2 that α7 receptors [39,40]. These experiments support the proposed mixed nicotinic–muscarinic modulation of ACh release in a cholinergic system related to glutamatergic transmission.

The picture is even more complex considering that metabotropic D2 autoreceptors and nicotinic α4β2 heteroreceptors can form heteromeric complexes [41]. At the interface of these aggregates, there is a direct or indirect involvement of the nicotinic β2 subunit. If this is a generalized mechanism, then muscarinic and nicotinic receptors might also form heteromeric complexes. If this is the case, then muscarinic receptors can be also modulated by conformational changes of nAChRs in the complex and upregulated with nAChRs.

Cholinergic–glutamatergic–DA system (II)

A third probable case is a variant of the previous case. Here, the negative feedback mechanism on ACh release is directly mediated by the same presynaptic α4β2 receptors (Figs. 2 and 5), similarly to the one described for muscle [42–46]. In muscle, two types of nAChRs regulate ACh release differently. Presynaptic muscle-type receptors positively regulate the release of ACh [44], a mechanism blocked by the muscle nAChR antagonist vecuronium and evident at high-frequencies of nerve stimulation. Another population of prejunctional nicotinic receptors negatively regulates ACh release at low frequencies of nerve stimulation. These receptors are probably of the α-bungarotoxin insensitive neuronal type likewise the α4β2 receptors [21,22,44–46]. A direct observation of a negative feedback mechanism in the nervous system as we propose here is lacking, as we saw α4β2 receptors are involved in positive feedback. All the experiments showing a positive feedback mechanism in the nervous system are based on acute administration of nicotine, so a dual response is possible, depending on nicotine concentration. In muscle, the negative feedback was only observed at low frequencies of nerve stimulation, or in other words under conditions of low ACh release.

Cholinergic–glutamatergic systems: addiction reinforcement and cognitive enhancement

Cholinergic afferents with presynaptic α4β2 receptors project to the hippocampus and make contacts with glutamatergic pyramidal cells [10]. Pyramidal cells are involved in the processes of long-term potentiation. This might produce addiction reinforcement in the VTA and in the hippocampus, cognitive enhancement. Hence, the same signals nicotine evokes in the VTA are registered in parallel in the hippocampus using the same neuronal circuitry, fixing

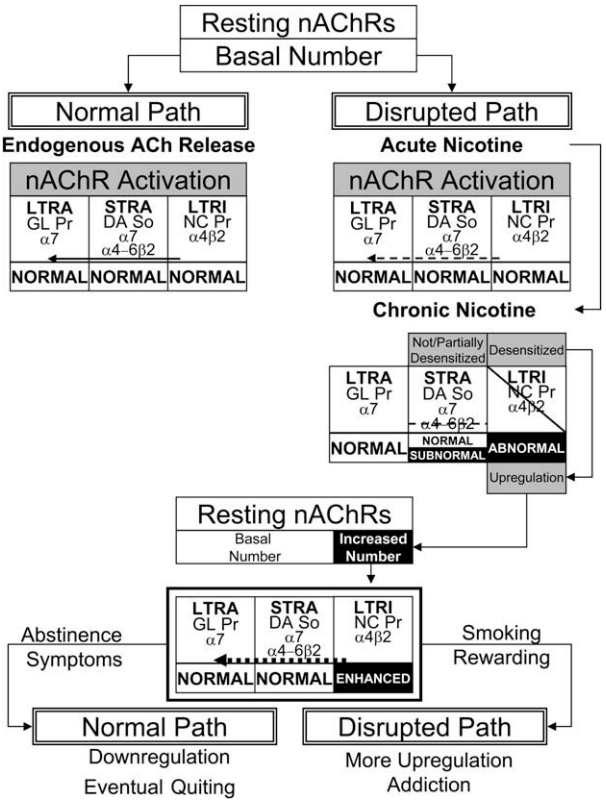

Fig. 5. Schematic representation of the mechanisms by which nicotine addiction is established by inhibition of a negative feedback that modulates a glutamatergic neuronal circuit (Case II). See text for a detailed explanation and Figs. 1–3 legends for abbreviations. The only difference with Case I (Fig. 4), is that in this instance nicotinic α4β2 receptors are directly responsible of the negative feedback on ACh release.

in memory the environment associated with smoking and pleasure.

Desensitization or blocking of α4β2 receptors and smoking

Nicotine self-administration in nicotine sensitized rats is reduced by pretreatment with α4β2 but not with α7 antagonists [47]. According to our proposal, in those conditions blocking of α4β2 receptors reduces nicotine self-administration by emulating nicotine, not by blocking its effects. α4β2 antagonists themselves cannot induce self-administration because they lack the dual property of activation/inactivation of nicotine. The same happens in nicotine sensitized rats, but in this case the α4β2 antagonist help in decreasing the number of upregulated and active α4β2, thus reducing the number of nicotine infusions taken.

It was proposed that smokers tend to enjoy the first cigarette of the day because nAChRs would not be in a state of prolonged desensitization [5,48], where desensitization is considered a cellular mechanism of nicotine tolerance. During nicotine abstinence at sleeping time and after upregulation, α4β2 receptors go back to the resting state allowing the activation of the negative feedback on ACh release thus reducing dopamine levels. Therefore, according to us, the enjoyment (rewarding effect) of the "first cigarette of the day" derives from nicotine inactivation of LTRI and transient activation of STRA. Non-smoking intervals during the day allow a subpopulation of α4β2 to return to the resting state. A persistent consumption of nicotine during the day is needed to keep most α4β2 receptors desensitized and inactive.

Exposure to nicotine: alterations in reward sensitivity and reward tolerance

Like other drugs of abuse, nicotine can increase the sensitivity to rewarding brain stimulation by lowering brain reward threshold (BRT) [49–51].

This enhanced reward sensitivity could be a consequence of nicotine based liberation of dopamine, as dopamine is involved in this process [52]. This could be the case for experiments where nicotine is directly injected [50] or self-administered [51]. With an acute dose of nicotine [50], it is plausible that the elevation of dopamine concentration is due to STRA. With self-administered nicotine two different results were obtained, depending on the available time rats have for nicotine self-administration, i.e. restricted (1 h) or extended (12 h). With 12 h, the basal BRT remained unaltered during the experiment. With 1 h exposure, the pre-exposure BRT downward shifted daily. Because this effect was observed starting on the ninth day of treatment, plausibly nAChR desensitization plays a role. The lower exposure to nicotine in 1 h exposed rats might have favored a subsequent desensitization of $\alpha4\beta2$ receptors in LTRI systems.

When nicotine is delivered chronically via subcutaneous osmotic mini-pumps [53], control and nicotine exposed rats do not differ in their BRT as with self-administration. Additionally, nicotine withdrawal, by means of pump extraction or precipitated by dihydro-β-erythroidine (DHβE, an $\alpha4\beta2$ specific antagonist), induces the elevation of BRT above pre-nicotine baseline.

These differences were explained based on the higher nicotine concentration delivered by mini-pumps compared to the obtained by self-administration [51]. The same authors proposed that volitional nicotine intake may trigger adaptations in reward circuitries different from those generated by passively administered nicotine, involving LTP and the glutamatergic system [7,51]. Our proposal is in agreement with their explanations. Under these chronic conditions (self-administered or passively injected) $\alpha4\beta2$ receptors are upregulated. When nicotine is present, a balance between a direct activation of nicotinic receptors present on DA neurons (elevation of dopamine baseline and reduction of BRT), and the activation of $\alpha4\beta2$ receptors on inhibitory GABA neurons (reduction of dopamine baseline and elevation of BRT) is possible. As nAChRs on DA neurons are not fully desensitized by nicotine, they can be continuously activated. On the other hand, $\alpha4\beta2$ on GABA neurons are desensitized and afterward upregulated, so this inhibitory system continues working with a mixed population of desensitized and active nAChRs. When nicotine is withdrew, the balance is lost because the inhibitory system is exacerbated as all $\alpha4\beta2$ on GABA neurons become active, with the consequence of a reduction of DA concentration and the elevation of the BRT.

However, in the self-administration experiments, where there is a difference between pre- and post-exposure BTR, a behavioral component is added that probably promotes the activation of a cholinergic–glutamatergic circuit [51]. This additional activation might explain not only the differences observed between pre- and post-exposure BTR, but also the posterior reinforcement of this circuit (LTP) responsible for the long-lasting low BTR. According to our description of LTRA, this glutamatergic circuit is not facilitated by acute nicotine unless concomitant with a behavioral activation of the afferent cholinergic fiber.

The increase of reward sensitivity due to acute nicotine exposure is blocked by mecamylamine [50] or reversed by DHβE [51,54], which is congruent with the blocking of STRA mechanisms. Yet, if we consider the proposed negative feedback role of $\alpha4\beta2$ in LTRI, nicotine and DHβE should have the same effect via desensitization or blocking, respectively. However, if DHβE displaces nicotine from its binding site, $\alpha4\beta2$ receptors exit the desensitized state promoted by nicotine, and enter earlier the resting state. Consequently, the negative feedback is gradually activated and dopamine concentration reduced.

Chronic nicotine and decrease of dopamine release

Nicotine increases dopamine in the NA, however, *chronic* nicotine reduces *basal* extracellular dopamine levels [55,56]. Nicotine self-administered rats have a slightly lower level of basal extracellular dopamine compared to their partners (nicotine yoked group), which receive simultaneously but involuntarily the same doses of nicotine [56]. Additionally, a reduction in the elevation of dopamine in the NA after a nicotine challenge (24–48 h after the last nicotine self-administration) was observed in both the self-administered group (112%) and the nicotine yoked group (121%) compared to controls (154%). These lower levels of DA in the NA were partially justified by an observed increase in dopamine reuptake as the result of chronic nicotine. Independently of this explanation, we do expect in our model a reduction in dopamine release after a chronic exposure to nicotine because LTRI systems in basal conditions are exacerbated by upregulation. The chronic nicotine treatment enhances via $\alpha4\beta2$ upregulation the negative feedback of the GABAergic circuit, and additionally in the nicotine self-administered rats, the $\alpha4\beta2$ – muscarinic negative feedback system associated to LTRA. According to this, it is expected a stronger reduction in dopamine levels in nicotine self-administered rats. However, if the basal dopamine reduction due to chronic nicotine is mainly the result of an enhanced reuptake, then the differences due to a lower dopamine release might be negligible, and this seems to be the case. However, the expected differences between control, nicotine self-administered and the nicotine yoked groups are reflected in the reduction of the elevation of dopamine after a nicotine challenge, where reuptake is not involved. In this case, nicotine self-administered rats had the lower increase in dopamine (112%) as expected.

Nicotine dependence and drugs used in smoking cessation

In our hypothesis nicotine dependence is central in triggering smoking addiction. Subsequently, behavioral components help in maintaining upregulation of $\alpha4\beta2$ receptors, albeit in advanced stages upregulation itself would not be the main factor in smoking addiction. However, once the decision of quitting has been made, nicotine biochemical dependence becomes an obstacle. Therefore, to overcome this problem and take the "normal path" instead of the "disrupted path" (Figs. 1, 3–5), any drug used for smoking cessation aid should have the same rewarding properties as nicotine (i.e. inhibit $\alpha4\alpha2$) without inducing $\alpha4\beta2$ upregulation. This seems to be the case for some of the current and emerging drugs.

Varenicline and other partial agonists [57–61], prevent nicotine from eliciting a maximal response. Varenicline has subnanomolar affinity only to $\alpha4\beta2$ nAChRs [62]. The antidepressant bupropion, another non-nicotine drug approved by the US FDA for the treatment of tobacco dependence, and some of its metabolites (hydroxybupropion) are also $\alpha4\beta2$ antagonists. Coincidently with our proposal, bupropion inhibits $\alpha4\beta2$ much more than $\alpha7$ receptors [63,64].

Mecamylamine is being tested as a possible treatment for nicotine dependence. Despite its classification as a non-selective nAChR antagonist, it is more potent blocking $\alpha4\beta2$ receptors compared to $\alpha7$ [39,40]. Moreover, mecamylamine has residual blocking effects on $\alpha4\beta2$ but not on $\alpha7$ receptors [40]. This slower dissociation would mimic the chronic effect of nicotine. Nevertheless, the blocking of $\alpha7$ and other non-$\alpha4\beta2$ receptors might

explain the initial increase in tobacco consumption after treatment with mecamylamine, and the need of transdermal nicotine co-administration [65]. The problem with nicotine is its propensity to upregulate $\alpha4\beta2$.

Nicotine dependence and schizophrenia

The putative involvement of nAChRs in schizophrenia was suggested by the high-percentage of smokers present in the schizophrenic population compared to the general population, 90% compared to 33% [66]. Schizophrenic subjects are particularly heavy smokers, even when compared with other psychiatric patients. This may reflect an attempt at self-medication of an endogenous neuronal deficit [67].

The dopamine hypothesis of schizophrenia proposes a cortical/subcortical imbalance. Subcortical mesolimbic DA projections are hyperactive resulting in D2 receptor hyperactivity and the observed positive (hallucinations and delusions) symptoms [68]. On the other hand, mesocortical DA projections to the prefrontal cortex (PFC) are hypoactive, resulting in hypostimulation of D1 receptors, cognitive impairment and the negative symptoms such as social withdrawal, anhedonia, apathy and paucity of speech [69]. Excessive dopaminergic neuronal activity is suggested to underlie schizophrenia positive symptoms because all typical antipsychotic drugs (D2 antagonists such as haloperidol and chlorpromazine) are effective in their treatment [70].

An important improvement to the classical DA hypothesis was the proposal that the deficiencies derived from the frontal dopamine hypofunctioning are primary in schizophrenia. Positive symptoms arise as the outcome of a secondary hyperfunction of dopamine in the striatum, due to the disinhibition of DA activity [71].

Dysfunctions in other neurotransmitter circuits are also involved. There is an evidence that the dopaminergic dysfunction is secondary to an alteration in glutamatergic (NMDA) transmission [72,73]. The NMDA receptor hypofunction hypothesis of schizophrenia is associated with the negative symptoms [74]. Low doses of NMDA receptor antagonists such as ketamine, can cause in normal subjects the negative and cognitive symptoms observed in schizophrenia [75]. Therefore, it seems plausible that the enhancement of NMDA transmission would be beneficial for the treatment of schizophrenia. Because direct agonist activation of NMDA receptors could be neurotoxic, an indirect approach to enhance NMDA function would be preferred. NMDA receptors have a glycine modulatory site (GMS) that must be occupied for glutamate to open the channel. For example, D-serine, a selective full agonist at the GMS, improved the efficacy of the atypical antipsychotics risperidone and olanzapine (but not clozapine) in the treatment of negative, positive, cognitive, and depression symptoms [76]; clozapine may exert its effects on negative symptoms directly by binding to the GMS [70].

The effects of nicotine in schizophrenics are complex. Nicotine elevates DA levels in the NA, critical in mediating the expression of positive symptoms [89]. Consequently, nicotine might exacerbate the positive symptoms directly via STRA and LTRA. However, LTRA (via NMDA receptors) is also involved in the glutamatergic hypofunction leading to the development of the negative symptoms, and secondary, to the positive symptoms as well. In this case, nicotine would be beneficial because it enhances glutamatergic transmission.

The picture is even more complex as post-mortem studies have shown a reduction in the density of $\alpha7$ receptors [77] a deficit that might have consequences in LTRA (but not STRA). One way to compensate the lower number of $\alpha7$ would be to activate for longer periods the remaining receptors. Nicotine consumption in schizophrenics transiently abolishes the negative feedback, keeping LTRA activated for longer periods also because $\alpha7$ are not desensitized.

However, $\alpha4\beta2$ upregulation enhances LTRI with stronger consequences in this case due to the reduced number of $\alpha7$ receptors. With LTRI enhanced with time both, the beneficial and detrimental effects on PCF and NA respectively, will be further inhibited. Nevertheless, it seems that the beneficial effects of nicotine prevail because schizophrenics do smoke. That is, enhancing periodically (with nicotine) the PCF glutamatergic pathway renders more benefits. Moreover, it is possible that nicotine based dopamine release in the midbrain might be negligible in an already exacerbated system. Under the glutamatergic hypothesis of schizophrenia, not only smoking would reduce the negative symptoms, but also in the long-term it would reduce the positive symptoms as well, if these are a secondary effect. The mechanism by which nicotine consumption seems advantageous in schizophrenics is similar to the proposed for agonist of the GMS in NMDA receptors, as both facilitate glutamatergic transmission. Furthermore, it was shown that clozapine treatment is able to modulate smoking [78,79]. This is congruent with the proposed mechanism of nicotine benefit in schizophrenia, as both nicotine and clozapine modulate glutamate transmission in the PFC in a similar way.

It was shown that atypical antipsychotics can produce an important increase of ACh release in the hippocampus [80]. Additionally, it was shown that these antipsychotics are nicotinic open channel blockers [81–83]. In a molecular modeling study [84], we suggested that the blocking properties of atypical antipsychotics were higher at $\alpha4\beta2$ receptors compared to $\alpha7$. A possible conclusion is that atypical antipsychotics by blocking $\alpha4\beta2$ with higher affinity compared to $\alpha7$, can reduce the need for nicotine by mimicking its desensitization effect. Therefore clozapine might modulate smoking via two different mechanisms in schizophrenic subjects, i.e. by direct positive modulation of glutamatergic transmission, and by inactivation of $\alpha4\beta2$ nAChRs.

Conclusions

We focused on the reinterpretation of the available experimental data and other models, and presented the relevant information needed to fundament a new hypothesis about nicotine addiction and smoking. The main aim was to find a way where desensitization, upregulation, and dopamine release enhancement, which are the most conspicuous biochemical effects of nicotine and usually ascribed to addiction, could be logically assembled to give an explanation. The idea that nAChR inactivation is the basis of nicotine dependence was proposed initially on logical grounds. We then proposed neuronal circuits in which nicotinic receptors are involved and matched the requirements of the hypothesis. In a previous hypothesis intending to explain nicotine addiction, Dani and Heinemann [85] proposed that chronic exposure inactivates nAChRs "leading to an increased number of nAChRs, which subsequently may lead to nicotinic cholinergic systems that are pathological". During abstinence, some nAChRs become active and "because of the increased number of nAChRs that have now become responsive in this pathological condition, some cholinergic systems other than the reward pathways become hyperexcitable to synaptically released ACh, contributing to the drive for the next cigarette". A problem with the hypothesis of Dani and Heinemann is that it is not explicit regarding the meaning of a pathological cholinergic system, or why hyperexcitability to released ACh should drive for continuous nicotine consumption. In our view the pathological cholinergic system is the upregulated negative feedback system on ACh release, and the drive for cigarette consumption is to reduce its effects.

Our proposal places emphasis on indirect regulation mechanisms, which are sometimes either difficult to observe directly in experiments involving isolated CNS regions, or are followed only in a short period. Consider that activation of nAChRs by acute administration of nicotine, can both activate and inhibit dopamine release, both in the short or in the long-term, and both locally or in distant regions [25]. On the other hand, chronic nicotine should have the opposite consequences via desensitization. These multiple effects might be also the basis for the different conclusions derived from *in vivo* [41] and *in vitro* experiments [86], which have opposite results with respect to the effects of nicotine in dopamine release.

Despite that this hypothesis seems to be congruent with the available data, some points have more and some have less supporting literature. Consequently it is necessary to give a brief delineation of the research we believe is needed to support or falsify our proposal.

Desensitization

An important point of the model is the degree of desensitization the nicotinic receptor types have after exposure to nicotine. We assumed that α4β2 is fully desensitized, or at least that there is a minimal nicotine (chronic) concentration where α4β2 is fully desensitized whilst α7 is not. Also, a general assumption based on the current literature, is that all nAChRs departing from the $(\alpha4)_2(\beta2)_3$ stoichiometry are less prone to desensitization than α4β2. Therefore, a better understanding of the desensitization properties of nAChRs under different nicotine concentrations is highly desirable to test these postulations.

Upregulation

Likewise with desensitization, a better comprehension of upregulation due to nicotine exposure is also needed. Even though it is clear that upregulation by nicotine is stronger for α4β2 receptors under chronic conditions, a deeper knowledge of the same process in the different kinds of nAChRs is highly important, especially *in vivo*. For example, it was shown that long-term nicotine treatment differentially regulates nAChR subtype expression and function, based on their localization in dopaminergic neuron populations [87]. Specifically, there is upregulation of the α6(non-α4)β2* subtype, but a loss of the α6α4β2* subtype with moderate nigrostriatal damage. Similarly, another study showed that chronic nicotine caused a decrease in α6* nAChRs in striatum but not in the superior colliculus, and additionally no changes in α3-containing nAChRs were observed in either region [88].

Basic model

A key line of investigation should address the veracity of the negative feedback mechanisms we proposed. At least, the "cellular" GABAergic negative feedback mechanism is well established. On the other hand, even though the available data is in agreement with our postulation on the α4β2 – muscarinic "molecular" negative feedback, further research is needed. An important issue is the internal modulation between these types of receptors. It would be desirable to know why muscarinic inhibition needs the activation of presynaptic nAChRs [35] and to investigate if nAChR α4β2 and muscarinic receptors do form heteromeric complexes as it happens with α4β2 and DA receptors [41]. It seems reasonable that both phenomena are interrelated. If this true, and upregulation is mediated by the increase of α4β2 receptors, then it is also expected a simultaneous upregulation of muscarinic receptors in these cholinergic fibers. The alternative negative feedback mechanism proposed and mediated by α4β2 alone, was observed in muscle but never in the nervous system. The experiments showing a positive feedback mechanism in the nervous system and mediated by α4β2 are based on acute administration of nicotine, so the study under chronic nicotinic exposure would reveal a different behavior. Perhaps α4β2 receptors in a "high-affinity state", as proposed to explain upregulation, would behave differently in this regards.

Drugs for smoking cessation

A conclusion of our model is that ideally, drugs used for smoking cessation should inhibit preferentially α4β2 nAChRs. This is accomplished by the partial agonist varenicline. However, a central problem is α4β2 upregulation, therefore it would be very important to know varenicline (and its present or future derivatives) propensity in this regard. Agonists are much more effective than antagonists (like DHβE) in upregulating α4β2 [14] and therefore varenicline might have an intermediate potency. In short, according to the present hypothesis a desirable property for a smoking cessation drug aside from blocking α4β2, would be to have a low or null ability to upregulate nAChR, as this characteristic allows the smoker to achieve downregulation without abstinence symptoms.

Conflicts of interest statement

None declared.

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