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Nicotinic Receptors Containing the $\alpha 7$ Subunit: A Model for Rational Drug Design

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Abstract

The neuronal nicotinic receptor has gained considerable recognition as a target, not just for combating drug addiction but also for treating a number of illnesses ranging from neurodegenerative diseases to psychotic disorders like schizophrenia. This recognition has led to a burgeoning field examining the receptor at all levels. A class of nicotinic receptors that contains the $\alpha 7$ gene product, apparently as a homomer, illustrates this multidisciplinary approach. Here, we review recent progress in our understanding of this class of receptors based on data from molecular, structural, physiological and patho-physiological studies. These studies have set the stage for rational drug design to combat disorders of the central nervous system. The studies also exemplify the cautious approach needed in developing CNS therapies and the importance of physiology in tempering drug design.

INTRODUCTION

Nicotine is a drug of abuse that places an enormous burden on the healthcare system. On an average, smokers are twice as likely to die of a number of illnesses, ranging from coronary heart disease and stroke, to lung cancer, than their non-smoking counterparts [1]. At the same time, the drug has been shown to be neuroprotective for diseases like Alzheimer's (AD) and Parkinson's [2–5] suggesting a potential therapeutic application for the drug. While the strategy of developing drugs that preserve the protective effects of nicotine without the addictive ones seems obvious, the wide-spread action of nicotine in the brain should give us pause lest we are left with unanticipated and unintended consequences.

A rational approach to drug design that targets the nicotinic system, must involve understanding of the bioavailability of the drug, drug targets- the nicotinic acetylcholine receptors (nAChRs), ligand binding kinetics, drug – receptor interactions based on structural data and, perhaps most importantly, the pharmacological, physiological, and pathological signaling mechanisms utilized by nicotine.

Here, we review the actions of this drug from view points ranging from its chemistry to its role in central nervous system (CNS) pathology. The aim of this review is not to delve into extensive details (for which the reader is referred to a number of excellent reviews), but to highlight the plethora of issues that present themselves in the process of combating CNS disorders and in the development of effective therapeutic strategies for these illnesses. We focus on a nAChR

sub-type containing the $\alpha 7$ subunit ($\alpha 7$ -nAChR), a predominant subtype in the brain, as an example to illustrate these points.

1. CHEMISTRY AND BIO-AVAILABILITY OF NICOTINE

When a cigarette is smoked the peak arterial concentration of nicotine rises to about 500 nM [6]. However, the drug tends to accumulate in various compartments in the body. For example, the concentrations of nicotine in amniotic fluid, placenta and in breast milk [7–9], as well as the brain [10] reach significantly higher levels than in the serum. Therefore while evaluating nicotinic effects at compartments like synapses in the brain, the actual nicotine exposure of these structures to the drug is not easily determinable.

The clearance rate of nicotine from to the blood stream depends on its degradation, mainly by the liver. The individual variability in nicotine clearance would determine the plasma nicotine concentration and, by extrapolation, smoking behavior. The major pathways that account for most of nicotine metabolism is shown in Fig. (1), based on data from urinary excretion [11].

1.1. The Cytochrome P450 System—The main site of nicotine breakdown is the liver, where most of nicotine is broken down into cotinine. The first step in this reaction is the conversion of nicotine to $\Delta^{1(5)}$ - iminium ion. This step is catalyzed by the Cytochrome P450 2A6 enzyme (CYP2A6). CYP2A6 is a ~50KDa protein that was characterized as the P450 enzyme with the major coumarin 7-hydroxylase activity [12,13]. The enzyme has a major catalytic role in the conversion of nicotine to cotinine and cotinine to 3'-hydroxycotinine (3'-HC). The levels of these metabolites are highly correlated with coumarin 7-hydroxylase activity from human liver microsomes as well as the CYP2A6 protein levels [14,15] and coumarin acts as a competitive inhibitor for nicotine oxidation.

A second site for the action of CYP2A6 is the hydroxylation of cotinine to 3'-HC. Therefore the levels of cotinine and 3'-HC act as good indicators of nicotine clearance. Indeed the clearance of oral nicotine correlates well with the plasma 3'-HC/cotinine ratios [16] and can be taken as a good indicator of CYP activity as well as act as a predictor of cigarette consumption in humans [17]. 8-methoxypsoralen, an inhibitor of CYP2A6 has been shown to facilitate smoking cessation, suggesting another target for therapeutics aimed at quitting the habit [18,19]. The availability of crystal structure for CYP2A6 at < 2 angstrom resolution [20] as well as the determination of characteristics of effective CYP2A6 inhibitors [21] will assist in the rational design of smoking cessation aids using these enzymes as targets.

While it appears that CYP2A6 is the major enzyme responsible for the C-oxidation of nicotine, patients lacking the enzyme still metabolize nicotine suggesting that other cytochrome P450 enzymes might be able to compensate. CYP2B6 shows significant nicotine oxidation activity while other enzymes CYP2D6 and CYP2E1 appear to have lesser roles in nicotine metabolism [22–25]. In a situation, analogous to the alcohol dehydrogenase, a number of polymorphisms have been described for CYP2A6. More than 25 different alleles have been reported in the literature for this enzyme (for a compilation of these alleles see [11]). These range from whole gene deletions to gene duplications showing increased enzyme activity. The occurrence of these alleles, coupled with data on 8-methoxypsoralen (see above) also raises the possibility that genetic variants could be good predictors of an individual's susceptibility to nicotine addiction.

1.2. Aldehyde Oxidase—In 1960 a second enzyme responsible for the conversion of nicotine to cotinine was characterized and termed to be an aldehyde oxidase [26]. It was later shown that this enzyme converts the $\Delta^{1(5)}$ iminium ion to cotinine suggesting that iminium oxidase might be a more appropriate name for this enzyme [27]. In the absence of this enzyme or in the presence of inhibitors, the iminium ion accumulates. Raloxifene, a selective estrogen

receptor modulator that is used in the treatment of postmenopausal women in order to reduce risks of osteoporosis and breast cancer, is a potent inhibitor of the aldehyde oxidase [28]. The compound acts as a non-competitive inhibitor for the enzyme with a K_i of 1–2 nM. Results also suggest that this effect is very specific for aldehyde oxidase as the compound has very little effect on xanthine oxidase, a closely related enzyme [28]. Whether this step in nicotine metabolism could represent a target for candidate drugs, remains to be seen.

1.3. UDP-Glucuronosyltransferase (UGT)—N-Glucuronidation of both nicotine and cotinine occur and have been shown to account for 3–4% and 9–17% of the metabolites excreted in the urine, respectively [29]. At the same time 3'-HC undergoes O-glucuronidation. Available evidence suggests that different UGTs are involved in the formation of N- and O-glucuronides [30,31]. While the existence of glucuronidation is unequivocal from a number of studies, the exact enzymes involved are less well characterized. Based on the inhibition profile using imipramine (which is UGT1A4 specific), propofol (specific for UGT1A9), and bilirubin (a substrate for UGT1A1), it was determined that UGT 1A4 might be the primary enzyme involved in the N-glucuronidation reactions with the other two playing a minor role [32,33]. On the other hand the existence of a specific enzyme for 3'-HC glucuronidation is yet to be determined. While polymorphisms of UGTs are known, their effects on nicotine and cotinine clearance are not clear.

1.4. Other Pathways for Nicotine Metabolism—Incoming nicotine gets converted to a number of other minor metabolites in the liver all of which, taken together, form about 15–20% of the metabolites found in the urine. Flavin containing monooxygenase 3 (FMO3) in liver microsomes converts nicotine to nicotine N oxide (NNO). Blocking FMO activity with methimazole, a selective inhibitor, prevented NNO formation from nicotine [34]. CYP2A6 in addition to its main role in the formation of cotinine and 3'-HC, also catalyzes the conversion of nicotine to nornicotine and cotinine to norcotinine, relatively minor metabolites in nicotine degradation. Lastly, a species specific N-methyltransferase activity resulting in formation of the nicotine isomethonium ion, has been reported from guinea pig and human liver cytosol but not from rat liver cytosol [35–37].

Alterations in the half life of nicotine using inhibitors of enzymes in the pathway forms one avenue in therapeutic strategies to combat nicotine dependence.

2. NICOTINIC RECEPTORS- TARGETS OF NICOTINE ACTION

Biological actions of nicotine are mediated by its ability to bind and open nicotinic nAChRs. These receptors are widely distributed in the central and peripheral nervous system and consist of multiple subtypes with differing pharmacologies. A total of 12 genes have been cloned and shown to code for the different nAChR subunits. These are classified into α subunits and β subunits, the α subunit being the ligand binding subunit. There are 9 α ($\alpha 2$ – $\alpha 10$) and 3 β ($\beta 2$ – $\beta 4$) subunits that comprise the nAChRs in the nervous system. The nAChR is a pentamer consisting of either five alpha subunits (homomeric) or combinations of an alpha and a beta subunit (heteromeric). The major homomeric receptor in the brain is made up of 5 $\alpha 7$ subunits ($\alpha 7$ -nAChRs) while the major heteromeric receptor consists of the $\alpha 4$ and $\beta 2$ combination ($\alpha 4\beta 2$ -nAChRs). Nomenclature of nAChRs is based on the prototypic receptor at the neuromuscular junction where the ligand binding subunit is the $\alpha 1$ subunit with one of the structural subunits that form the pentamer being the $\beta 1$ subunit. The pentameric receptor, viewed from the top, is arranged in a doughnut like shape with the subunits surrounding a central pore which is the ion channel through which receptor signals are transmitted. Binding of the agonist, nicotine or acetylcholine, the endogenous transmitter, results in a conformation change leading to the opening of the ion channel. The receptor pore forms a cationic channel that allows the influx of sodium ions and, to a lesser extent, calcium ions. The current due to

calcium influx through the pore forms about 1–5% of the total ion flux but is extremely important in transducing receptor signals. This is especially true for the $\alpha 7$ -nAChRs which have a high calcium permeability (see below).

2.1. Ligand Binding Properties of nAChRs—nAChRs can be considered as prototypic allosteric proteins, with the receptor existing in several interconvertible conformational states [38], varying in their ligand affinities and their open/closed state. The ligand binding site and the channel domain are separate, thus leading to the possibility that the two can be independently modified. Ligand (in this case nicotine or acetylcholine, the endogenous ligand) binding stabilizes one or more receptor states resulting in altering the net behavior of the nAChRs. Using an adaptation of the Monod Wyman Changeux (MWC) model for allosteric interactions [39] given by the equation below, one could arrive at a simplistic model for receptor kinetics transitioning between states A (open) and state B (closed). In the equation, A , is the fraction of receptors in the open state, L is the isomerization constant between states, K_a and K_b are the respective dissociation constants for nicotine for the two states, n is the number of ligands bound.

$$\bar{A} = \frac{1}{1 + L - \left[\frac{1 + [Nic]/K_b}{1 + [Nic]/K_a} \right]^n}$$

What is also known about most ligand-gated ion channels is that longer exposure to agonists can drive the receptors to a third state, the desensitized state, which represents a closed conformation but with a high affinity for the agonist Fig. (2). The receptor in the closed state (R) sequentially binds two nicotine molecules leading to the opening of the ion channel (O). The agonist bound state now is driven to D, the desensitized form. From D the receptor returns to the unliganded form or possibly, re-traverses the open state. While this model provides a brief description of ligand binding and channel opening, in reality, the process is likely to be much more complex.

Kinetic models from point mutations followed by single-channel analyses suggest the existence of multiple sub-states e.g. see [40]. Indeed cryo-EM studies on the ligand bound conformation of the *Torpedo* nAChRs suggests the possible existence of at least 10 ligand bound states [41]. This indicates that these states might exist as a continuum of conformations rather than discrete states as depicted below. However, for the understanding of the receptor in terms relevant to physiological and pathological signaling mechanisms, the 3 kinetic states described here might suffice, though the contention is open to debate.

From a therapeutic view, the idea of stabilizing the channel in the open, closed, or desensitized state, in order to control synaptic signaling is an attractive one and one that has considerable promise in the treatment of illnesses involving $\alpha 7$ -nAChRs. This possibility is currently being exploited (see below).

2.2. Information from Site-Directed Mutagenesis—nAChRs belong to the family of Cys-loop proteins that have a signature sequence of 13 amino acids flanked by cysteines in the N-terminal region. The cysteines at the two ends are linked covalently to each other forming a closed loop that couples the ligand binding domain from the channel domain.

Hydropathy plots indicate four transmembrane domains for each subunit, given by M1–M4 in Fig. (3). The M2 loops (one from each subunit) form the channel domain. The next transmembrane segment (M3) is separated from the last transmembrane domain by a large cytoplasmic loop. This loop is the target of regulation by intracellular second messengers.

Regulation of receptor function by the phosphorylation of serine/threonine, as well as tyrosine, residues occurs at this loop [42].

Considerable insight into the kinetics and allosteric mechanisms of nAChRs has been gleaned by studies using site-directed mutagenesis. Distinct modular organization of the receptor was shown using a chimeric protein which contained the ligand binding domain of $\alpha 7$ -nAChR and the channel domain of the serotonergic 5HT3 receptor. The chimeric receptor channel now was activated with nAChR agonists and blocked by competitive antagonist for the receptors [43]. Substitution of a single amino acid in the M2 region of $\alpha 7$ -nAChRs (L247T) altered the desensitization kinetics of the receptors [44,45]. The mutation was shown to decrease the rate of desensitization of the receptor. In addition, the channel exhibited an additional high affinity conduction state at low agonist concentrations suggesting, as depicted in the state diagram above, the possibility that the receptor could traverse directly from a desensitized to an open state, circumventing the closed, unbound conformation. A third characteristic of the mutant, consistent with the allosteric nature of the transitions is that they exhibit a significant number of spontaneous openings even in the absence of the agonist [46], not predicted by the Koshland induced-fit hypothesis [47]. Paradoxically, the L247T mutant now exhibited an altered pharmacology as well, considering that the point mutation was located in the M2 channel domain [46]. The nAChR competitive antagonist dihydro- β -erythroidine (DH β E) now functioned as a full agonist while another competitive antagonist of $\alpha 7$ -nAChRs, methyllycaconitine (MLA) did not. Once again this is consistent with the state diagram above by postulating that DH β E inhibits the receptor activity by locking the receptors in the desensitized state while MLA's antagonism comes from stabilizing the closed state conformation. The gating of nAChRs, i.e. the agonist-mediated transition to the open state occurs in microsecond time scales demonstrating a very efficient coupling between the two domains [48]. This was demonstrated to require specific residues in the cysteine loop [49] by using chimeras between two fast gating channels the $\alpha 7$ -nAChR and the anionic glycine receptor (GlyR). Inefficient coupling between the two resulted in slow activation rate which was overcome by reinstating two specific cys-loop amino acids that belonged to the receptor contributing the channel domain [49].

A key feature of the $\alpha 7$ -nAChR channel is its high calcium permeability [50,51]. In addition the receptor uses multiple calcium amplification pathways to very effectively raise intracellular calcium levels [52–54]. Indeed much of the physiological effects of the receptor are due to its ability to mediate calcium-dependent signal transduction in neurons. Another key feature of the receptor consistent with the allosteric model is its modulation by extracellular calcium concentrations. The receptor currents are potentiated by increasing external calcium concentrations [55]. Mutations in the canonical EF-hand domain located at the N-terminal portion of the receptor alter this calcium-mediated potentiation [56]. nAChRs are also regulated by covalent modification of amino acids in the cytoplasmic loop; for example see [57]. While not examined in detail there is evidence for changes in $\alpha 7$ -nAChR function by phosphorylation. Neuregulins, a family of neuronal proteins that regulate tyrosine kinase activity, cause rapid internalization of $\alpha 7$ -nAChRs *via* tyrosine phosphorylation by an EGF receptor related kinase [58]. Interestingly, a naturally occurring mutation in the human $\alpha 7$ -nAChR (G423S) caused rapid desensitization of receptor currents [59]. Other calcium-dependent serine/threonine kinases also have been shown to affect $\alpha 7$ -nAChR desensitization [60]. These studies show yet another process that can be therapeutically exploited.

2.3. Insights into the Structure of $\alpha 7$ -nAChRs—Much of what we know about the structure of nAChRs comes from the study of the receptor at the neuromuscular junction and its counterparts in electric organs of fish. Inherent problems in crystallizing membrane proteins has necessitated a more circuitous and tedious process trying to determine the structure of these receptors [61–64].

Cryo-EM examination of receptor arrays that are present in electric organs of fish like the electric eel and the *Torpedo marmorata*, revealed some of the finer structure of the receptor up to a resolution of about 4 Angstroms [62–65]. A serendipitous finding brought our understanding of the ligand binding domain structure of the receptor closer to or atomic resolution. It was discovered that the sea mollusk *Lymnaea stagnalis* has glial cells that release and acetylcholine binding protein (AChBP) that consists of the nAChR ligand binding domain in a soluble form and hence amenable to crystallization [66,67]. Combining these results with those elucidated by Cryo-EM a clearer picture of the nAChR structure has emerged. Considering that the nAChRs on neurons are likely to be similar, much of the structural information obtained from these studies is likely to be true for the receptors on neurons as well. Indeed the AChBP has much greater homology to the $\alpha 7$ -nAChR, a major nAChR in the brain [66,68].

Following is a brief summary of what is known about nAChR structure from pioneering studies on the muscle type receptor and extrapolation to what the structure of $\alpha 7$ -nAChRs might be from the fact that much of the receptor properties are conserved across subtypes. The nAChR channel structure deduced from the *Torpedo* receptors shows a large channel spanning a length of about 160 angstroms normal to the membrane plane. A 20 angstrom ligand-binding domain forms part of a central vestibule and contains two agonist binding sites [65]. Each subunit in this domain is organized around two sets of β -sheets joined *via* a disulfide bridge that forms a part of the cys-loop [68].

Loops M1–M4, as shown in Fig. (3) are organized as α -helices in such a fashion that the M2 helix from all five subunits form a ring lining a narrow water filled pore of the ion channel. Deep within the ion pore is a ring of leucine residues that form a hydrophobic constriction in the pore which form the ‘gate’ of the channel. In the absence of the agonist this ‘gate’ is closed and prevents ion flux through the channel. When agonist binds to the channel, as evidenced from elegant studies performed by trapping the receptor in an agonist bound state [41], the α -subunits M2 helices change their configuration widening the pore and thus opening the ‘gate’. Cations now permeate the channel resulting in membrane depolarization.

Some evidence for the structure of $\alpha 7$ -nAChRs comes from studies comparing simulated receptor conformation based on data from the receptor in unliganded state and in the presence of multiple bound ligands [69–71]. Key findings from these studies are as follows. The apoprotein and the receptor in the presence of an antagonist (d-tubocurarine; dTC) show a considerable amount of asymmetry presumably resulting in a narrow pore. However, the asymmetry in the two cases arises from different motions of the ligand-binding domain. The apoprotein shows an asymmetric but tightly packed subunits whereas in the dTC bound asymmetry, the packing is much looser. In either case one would postulate that the resultant narrow pore would be impermeable to ion flow across the membrane. The binding of ACh results in a loosely packed but symmetric arrangement and a resultant larger pore. Binding of calcium, the positive allosteric modulator, results in a looser conformation. Once again these structures presumably lead to a wider, permeant pore. However, in spite of the fact that the receptor is a homomer containing five ligand binding subunits, the Hill coefficient (n_H) was shown to be closer to 2; e.g see [72] suggesting a stochastic binding of two agonist molecules was sufficient to open the channels. Though there is no convincing evidence for functional non-equivalence of the binding sites, it remains a possibility that differential binding of agonists to two of the five sites might confer differential properties or that the channel opens with partial occupancy. There is also a possibility that receptor desensitization is proportional to occupancy and that low receptor occupancy by the ligand might result in a more tonic, slowly desensitizing current [73,74]. Such an idea would be consistent with a very slow rising calcium signal upon receptor activation, not completely explainable by downstream amplification [75].

3. SIGNALING BY $\alpha 7$ -nAChRs

As discussed above, a key feature of the $\alpha 7$ -nAChRs is their high permeability to calcium [50,76] comparable to that of the NMDA class of glutamate receptors, the gold standard for high calcium permeable ligand-gated ion channels. Perhaps, more importantly, the receptor is very efficient at raising intracellular free calcium levels ($[Ca]_i$) [53,54,75]. This efficiency arises from the fact that calcium signals through the receptors are amplified by subsequent activation of voltage-gated calcium channels as well as calcium release from the endoplasmic reticulum (ER). The latter means of amplification happens *via* a process called calcium-induced calcium release (CICR), where flux through the receptor opens ryanodine receptors (RyRs) on the ER. RyRs are ligand-gated ion channels that use calcium as the ligand. As these receptors have a relatively low affinity for calcium (~ 10 – $100 \mu M$), it implies that the $\alpha 7$ -nAChRs and RyRs must be in close proximity to each other. This is because most cells have a very high calcium buffering capacity (\sim equivalent of 2 mM BAPTA [77]) which rapidly attenuates calcium concentrations such that $[Ca]_i$ drops to $1 \mu M$ or less about 250 nm from the influx site. In addition, the calcium signaling can be further amplified by release through inositol trisphosphate receptor (IP₃Rs) on the ER, thus resulting in a calcium cascade that propagates along the entire cell [53].

Efficient modulation of $[Ca]_i$ results in the activation of a host of calcium-dependent signaling cascades that mediate short- and long-term effects of $\alpha 7$ -nAChRs. Fig. (4) shows a simplified diagram illustrating major pathways mediating signal transduction upon $\alpha 7$ -nAChR activation. There is evidence for the activation of almost all calcium-mediated second messengers. This complex cascade of downstream enzymes mediate changes in synaptic strength and plasticity affecting a number of effects ranging from addiction to attention. A major endpoint of this signaling that results in a long-term change in gene expression and synaptic strength is the activation by phosphorylation of cyclic AMP response element binding protein (CREB). A number of calcium-activated enzymes lead to CREB activation. The activation of VGCCs by $\alpha 7$ -nAChRs leads to production of cAMP [78]. This second messenger can activate protein kinase A leading to CREB phosphorylation, activation of the mitogen-activated protein kinase (MAPK) and a feedback regulation of VGCCs and potentially the $\alpha 7$ -nAChRs [57]. MAPK further amplifies the activation of CREB; Fig. (4).

A second important cascade starts with the activation of calcium/calmodulin-dependent protein kinase II (CaMKII) which converges on CREB activation as well as phosphorylates a number of substrates. In the postsynaptic neuron, CaMKII regulates glutamate receptor on the postsynaptic neuron leading to the facilitation of long-term potentiation (LTP), thought to be the cellular correlate of learning and memory [79–82]. Similarly the role of protein kinase C (PKC) has been documented as well [83–87].

What these data tell us is that $\alpha 7$ -nAChRs are indeed positioned and capable of effectively altering neuronal functions and synaptic strength in the CNS.

One long-term consequence of $\alpha 7$ -nAChR-mediated calcium changes is the regulation of neuronal survival in the CNS. Nicotine, acting *via* this receptor, can be cytotoxic to developing neurons and adult progenitor cell [88–90] while being neuroprotective for differentiated neurons [91,92]. Both these effects of the receptor activation are mediated *via* changes in $[Ca]_i$. This apparent paradox can be explained by the finding that calcium signaling is coded by narrow spatial and temporal patterns. Immature and undifferentiated neurons have poor calcium buffering capabilities thus causing large and long-lived changes in $[Ca]_i$ resulting in apoptotic implications for the role of this receptor in neurodegenerative diseases like Alzheimer's disease (AD) and Parkinson's (PD). This is discussed below.

Another major function of $\alpha 7$ -nAChRs is their modulation of transmitter release. These receptors, also present on terminals of heterologous transmitter systems potentiate the release of glutamate [52,75,93], and GABA [94–97] in the brain, in addition to other transmitters. In a hippocampal synapse this presynaptic release mediated by $\alpha 7$ -nAChRs can mediate synaptic transmission in the absence of presynaptic action potentials (AP) suggesting that activation of these receptors can actually usurp normal signaling pathways in the brain [52,75]. Another important way in which $\alpha 7$ -nAChRs modulate synaptic function is by the activation of GABAergic interneurons [94,98,99].

The presence of $\alpha 7$ -nAChRs in non-neuronal cells has remained a puzzle over the years. The receptor is expressed in a wide range of cell types throughout the body [100] where they might serve different functions, like modulation of cell death, migration and signaling, mainly by their ability to modulate calcium levels. For example in T-lymphocytes, nAChR-mediated calcium change is correlated with T-cell anergy [101]. Nicotine exposure increases the proliferation of vascular endothelial cells [102], stimulates the expression of nitric oxide synthase [103] and has a net positive effect on angiogenesis [104]. Increased vascularization by nAChR-activation has implicated a role for the drug in tumor promotion.

An unusual finding is that non-neuronal cells in the brain, especially astrocytes, appear to possess $\alpha 7$ -nAChRs [53,100,105,106] suggesting that the effects of the receptor might be much more widespread than discussed above. Taken together, the wide distribution of $\alpha 7$ -nAChRs, and the efficiency with which they modulate transmitter release and calcium signaling, makes them likely candidates to mediate the effects of nicotine in addiction and neurodegenerative diseases. At the same time, their localization throughout the body makes it unlikely that one could develop strategies to specifically target these receptors in a particular area.

4. NICOTINE ADDICTION

The fact that nicotine is addictive is not in question, at least among neurobiologists. The mechanism of addiction, however, remains an open question. More than five decades ago James Olds discovered that electrical stimulation of certain regions of the brain resulted in positive reinforcing effects, and that rats could be trained to self-stimulate as long as electrodes were placed in these regions e.g. see [107,108]. This ‘pleasure drive’ was strong enough to override the effects of hunger as well as sexual stimulation [107]. The idea that these areas, traced to the mesolimbic dopaminergic pathway, could be involved in the positive reinforcement of drug addiction, came later with studies demonstrating that animals could be trained to self administer drugs of abuse when delivered to the same mesolimbic system [109]. Since then much of addiction research has been focused on the modulation of dopamine release [110–112]. $\alpha 7$ -nAChRs have been shown to be located on glutamatergic terminals of the ventral tegmental area (VTA), the starting point of the mesolimbic dopaminergic system [113]. There is some evidence that the $\alpha 7$ -nAChRs enhance glutamate release and suppress GABA release on to dopaminergic neurons [114,115]. This would argue for the direct action of $\alpha 7$ -nAChRs on the mesolimbic reward system. At the same time nicotine also modulates dopamine release [116–118]. Taken together, these results suggest a direct action of nAChRs in general, and $\alpha 7$ -nAChRs in particular, on the brain reward system thus providing a link between the drug and addiction.

It must be noted that the mesolimbic dopaminergic pathway is not the only one likely to mediate addictive effects. It is being recognized that addiction is a non-linear process, involving homeostatic dysregulation rather than stimulation or inhibition of specific pathways [119, 120]. Thus $\alpha 7$ -nAChRs in many other brain areas could influence the addictive nature of nicotine. This viewpoint makes sense as the number of possible drugs that are addictive and the few instances of cross-addiction make it unlikely that there would be a single reward

pathway. However, it is likely that addiction does involve the mesolimbic dopaminergic pathway, though its alteration might arise by inputs from a number of brain areas.

Studies with the nAChR gene knockouts (KOs) suggest differential roles for different receptor subtypes. While $\beta 2$ -KOs result in the loss of nicotine self administration [121], $\alpha 7$ -gene KOs might show a decrement in nicotine withdrawal symptoms [122,123]. These results further emphasize the notion that nicotine addiction involves a complex interplay of various nAChR subunits.

5. $\alpha 7$ -NICOTINIC RECEPTORS IN NEURODEGENERATIVE DISEASES

Nicotine has been shown to positively modulate a number of brain functions linked to cognition. It improves attention, arousal, learning, learnt discrimination, and memory functions [124–127]. Many of these effects of the drug are mediated by $\alpha 7$ -nAChRs activated by the drug. These effects have been linked to learning as well as in neurodegenerative diseases. A key finding over the years has been the role of $\alpha 7$ -nAChRs in neuronal survival [88–92] increasing the plausibility of a role for these receptors in neurodegenerative diseases.

5.1. Nicotine and Alzheimer's Disease—Nicotine has been shown to increase memory and attention in normal humans [128]. In AD the drug has been shown to improve memory deficits. This idea is supported by epidemiological data suggesting that incidence of AD among smokers is significantly less than in non-smokers [129]. The cholinergic hypothesis for AD has been prevalent for a long time based on the finding that loss of basal forebrain cholinergic neurons is one of the early symptoms of AD. This led to the use of acetylcholine esterase (AChE) inhibitors for treatment of the disease [130]. The results from this line of therapy have been disappointing, thus undermining the idea as a whole. Upon reflection, however, these findings are not contradictory to the cholinergic hypothesis. The efficacy of the AChE inhibitors, whose function is to increase the lifetime of the transmitter in the extracellular space, depends on the presence of cholinergic projections. If these are the earliest neurons to die, as suggested, the loss of projections would render inhibition of AChE ineffective. More recent data suggests that nAChR agonists and antagonists might be a better, more effective, therapeutic approach to the disease. Some attempts at drug development based on this idea have been made. This is summarized in the next section.

A feature of $\alpha 7$ -nAChRs is their modulation by the beta amyloid 1–42 peptide ($A\beta$), the key component of plaques found in the brain of AD patients. Some evidence exists showing that $A\beta$ by activating $\alpha 7$ -nAChRs, can modulate the MAPK pathway and CREB activation [131]. At the same time the receptor is downregulated in AD brains [132]. Further, in transgenic mouse models of AD, nicotine *via* the MAPK pathway also increases the hyperphosphorylation of the *tau* protein, the cause of neurofibrillary tangles, which is a part of the AD pathology as well [132,133]. Thus the exact mechanisms which mediate the enhanced cognitive functions seen upon nicotine treatment remains unclear.

5.2. Parkinson's Disease—Nicotine has been shown to be protective against PD as well. In PD, there is a specific loss of the dopaminergic neurons of substantia nigra, which provide inhibitory control to the neurons of the striatum. Once again the role of nAChRs is likely to be complex, involving differential modulation of a number of pathways. In mouse models of PD, where selective lesions of dopaminergic neurons were made by injection of 6-hydroxy dopamine (6-OHDA) selectively into the striatum or the substantia nigra, the levels of a number of nAChRs showed dramatic decline though the $\alpha 7$ -nAChR levels remained unchanged [134]. In patients with PD there is again a selective increase in $\alpha 7$ -nAChRs while levels of heteromeric nAChRs decline [135,136]. $\alpha 7$ -nAChRs have been shown to trigger an anti-

inflammatory pathway in brain microglia [137]. The activation of the receptor can suppress the inhibition of pro-inflammatory transcription factors NF κ B and c-myc [138].

In neurons, dopamine is oxidized by monoamine oxidases (MAOs). One class of MAO; MAO-B, oxidizes dopamine and various primary and tertiary amines to their corresponding aldehyde and free amines, resulting in the release of hydrogen peroxide a source of free radicals. The oxidation of dopamine to dihydroxy phenyl acetic acid, *via* a series of reactions, generates a number of reactive oxygen species (ROS). As the brain has a more limited capacity to clear ROS than other tissues, these species can trigger a cytotoxic cycle, wherein in the presence of solvated Fe(II) and H₂O₂, the toxic 6-OHDA is formed (Fenton Reaction). The 6-OHDA, in turn, is able to mobilize more Fe(II) from stored forms of iron in proteins, thus propagating neurotoxicity and neuronal death. Nicotine has been shown to be neuroprotective by blocking MAO activity, thus acting as a protective antioxidant [139].

Thus there are both epidemiological, as well as potential mechanistic bases, for the protective role of nAChRs in neurodegenerative diseases and it is likely that α 7-nAChRs play an important role.

6. α 7-nAChRs IN SCHIZOPHRENIA

The involvement of α 7-nAChRs in schizophrenia is backed up by a copious amount of literature. The schizophrenic population shows a much greater incidence of smoking than the general population e.g. see [140]. In addition, patients show a comparatively poor level of auditory sensory gating and smoking helps compensate for this deficit. These findings led to the examination of nAChRs in schizophrenia. [141]. Auditory gating is measured as changes in a specific peak in EEG recordings. This response, known as the P50 auditory evoked response, is seen about 40–80 ms after the presentation of the auditory stimulus. In normal population presentation of two stimuli closely spaced in time (~500 ms) results in the attenuation of the P50 response to the second stimulus. This relative suppression of the P50 response is an indicator of sensory gating. There is much less suppression of the P50 evoked response in schizophrenics [142], leading to the idea that defects in this process contribute to schizophrenic symptoms. Consistent with epidemiological data, smoking restores, to a large extent, the P50 ratios in schizophrenics [143] implying a role for nAChRs in this process. A number of studies indicate that P50 deficits show significant correlation with the level of α 7-nAChRs in the brain [144]. Further, infusion of α 7-nAChR antagonists decrease P50 ratios (i.e. response to the second tone not suppressed) while agonists increase them [145]. This effect is mimicked by the atypical antipsychotic, clozapine, in a manner consistent with its effects being *via* α 7-nAChRs [145]. Linkage analyses showed that the P50 changes were mapped to the chromosomal locus 15q13-q14 [146]. The α 7 gene lies within this locus thus providing good correlation between α 7-nAChRs, P50 deficits and schizophrenia [147]. These results also suggest that therapeutic interventions based on modulating α 7-nAChR function might be useful in the treatment of certain schizophrenic symptoms as well.

7. THERAPEUTIC STRATEGIES BASED ON nAChRs

The multifarious role of nAChRs in various physiological and pathological processes and their subtype specific actions make them ideal targets for rational drug design. At the same time, the ubiquitous distribution of nAChRs warrants a more cautious approach to therapeutics based on these receptors for fear of potential side effects. The potential importance of α 7-nAChRs in neuro-degeneration has led to the search of specific drugs targeting this receptor subtype. One approach that has significant potential is based on rational design comes from modeling data based on nAChR structures taking advantage of different local binding properties of different subtypes of the receptors [148].

A second approach, based on chemical modification of known ligands, has yielded some success as well in the search for potential subtype specific drugs. The marine worm toxin anabasine has been shown to act as a specific ligand for nAChRs. The benzylidene derivatives of this toxin have been shown to have a high degree of selectivity for $\alpha 7$ -nAChRs. Two of these derivatives, (3-(2,4-dimethoxybenzylidene) anabasine (DMXBA; GTS-21) and 4OH GTS-21 have specific agonistic properties for $\alpha 7$ -nAChRs [149–151]. This compound is now at Phase II trials for the treatment of schizophrenia [152]. Tropisetron, an anti-emetic, which is an agonist for serotonin receptor subclass (5-HT₃), is also a partial $\alpha 7$ -nAChR agonist. This drug attenuates P50 gating deficits [153]. (R)-(-)-5'-Phenylspiro[1-azabicyclo[2.2.2]octane-3,2'-(3'H)furo[2,3-b]pyridine (PSAB-OFP) is another agonist shown to be selective for $\alpha 7$ -nAChRs [154,155]. Marrero *et al.* [156] showed that 2-(3-pyridyl)-1-azabicyclo[3.2.2]nonane (TC-1698) has significant neuro-protective action *via* its ability to act as an $\alpha 7$ -nAChR agonist. Varenicline (6,7,8,9-Tetrahydro-6,10 methano-6Hpyrazino [2,3-h] [3] benzazepine)), a compound based on the structure of cytosine, an $\alpha 4\beta 2$ agonist is a complete agonist at $\alpha 7$ -nAChRs and is a drug now approved for smoking cessation treatment [157].

Allosteric modulators of $\alpha 7$ -nAChRs provide yet another target for rational drug design. A novel positive allosteric modulator of $\alpha 7$ -nAChRs, 1-(5-chloro-2,4-dimethoxyphenyl)-3-(5-methyl-isoxazol-3-yl)-urea (PNU-120596) [158], has been shown to increase the mean open time of the receptor channels and also increases the efficiency of $\alpha 7$ -nAChR-mediated changes in calcium levels [75,158].

The above examples of specific targeting of compounds to the $\alpha 7$ -nAChRs serve to illustrate the current progress in drug design based on nAChR subtype specificity. In addition to the $\alpha 7$ -nAChRs, other subtype specific agents have shown significant promise for the treatment of a number of potential pathological conditions (for review see [159]). This dramatic increase in interest in nAChR-based drugs further serves to illustrate the importance of these receptors in the modulation of various physiological and pathological conditions in human CNS.

CONCLUSION

It is a truism, but one often underappreciated, that a close interaction between physiology, chemistry, and pharmacology is necessary for successful therapeutic strategies. What is not clearly resolvable is the relative emphasis that needs to be placed on the extent of our knowledge in each of these disciplines. On the one hand, it is impractical and highly imprudent to wait until we know all there is to know (if that is definable) about a disease before developing drugs to combat it. On the other hand, one has to deal with the possibilities of unintended consequences that always accompany drugs based on incomplete information, warranting a more cautious approach. Nowhere is this conundrum clearer than in the case of nAChRs, a class of neurotransmitter receptors ubiquitous in their distribution in the brain and elsewhere, and one that modulates a huge range of physiological functions. Research on a major subtype of this receptor, the $\alpha 7$ -nAChRs, illustrates an approach by combining information from all these disciplines to arrive at strategies for rational drug design. The important roles played by this receptor in addiction and neurodegenerative diseases illustrates the promise of these drugs as a new set of weapons in our arsenal for the treatment of these devastating disorders.

Data from both metabolism of nicotine and structure, function, and signaling by nAChRs, suggest a number of possible sites of interest for rational drug design. Selective alterations in nicotine levels, as well as changing receptor signaling by either altering kinetics or signaling efficacy could result in drugs specific for one or the other consequences of nAChR activation. Though yet to be realized, these approaches have the realistic promise of effective therapies targeted at what are, perhaps, the most intractable of CNS disorders.

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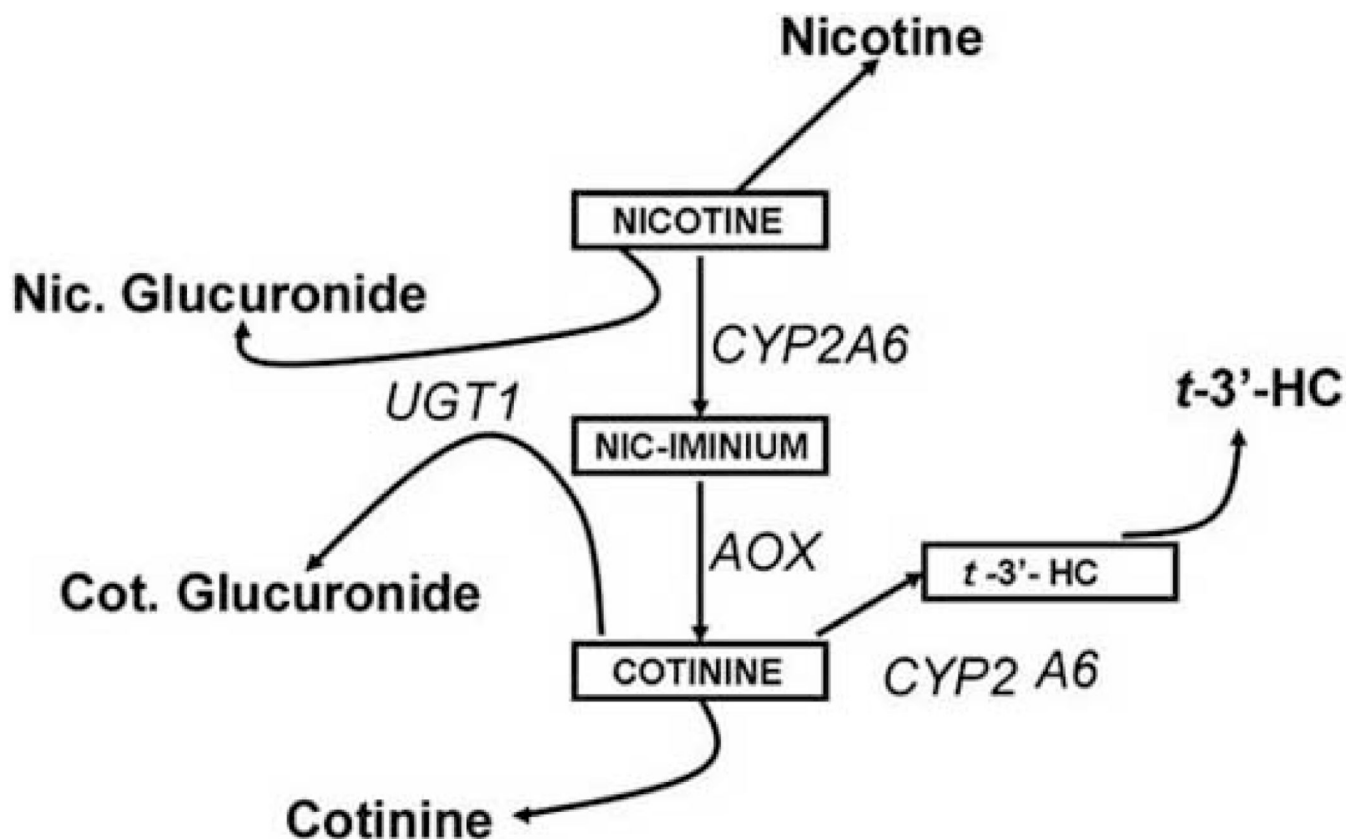


Fig. (1). Pathways for nicotine degradation

Once absorbed in the bloodstream nicotine is degraded, in the liver, by a variety of enzymatic pathway to give rise to identifiable and measurable reaction products. Abbreviations: *CYP2A6* - cytochrome P450 2A6; *AOX* - Aldehyde oxidase; *UGT1* - UDP-glucuronosyltransferase; *t'-3'-HC* - 3'-hydroxy cotinine.

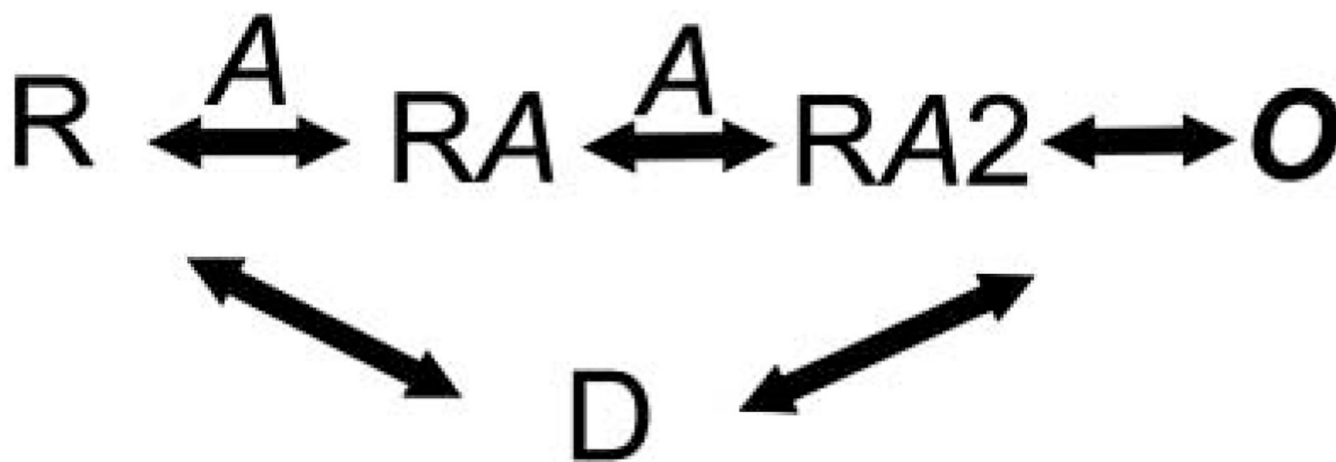


Fig. (2). A simple kinetic model for receptor activation

R- unbound state of the receptor; **A** – agonist; **O**- open state; **D** – desensitized state.

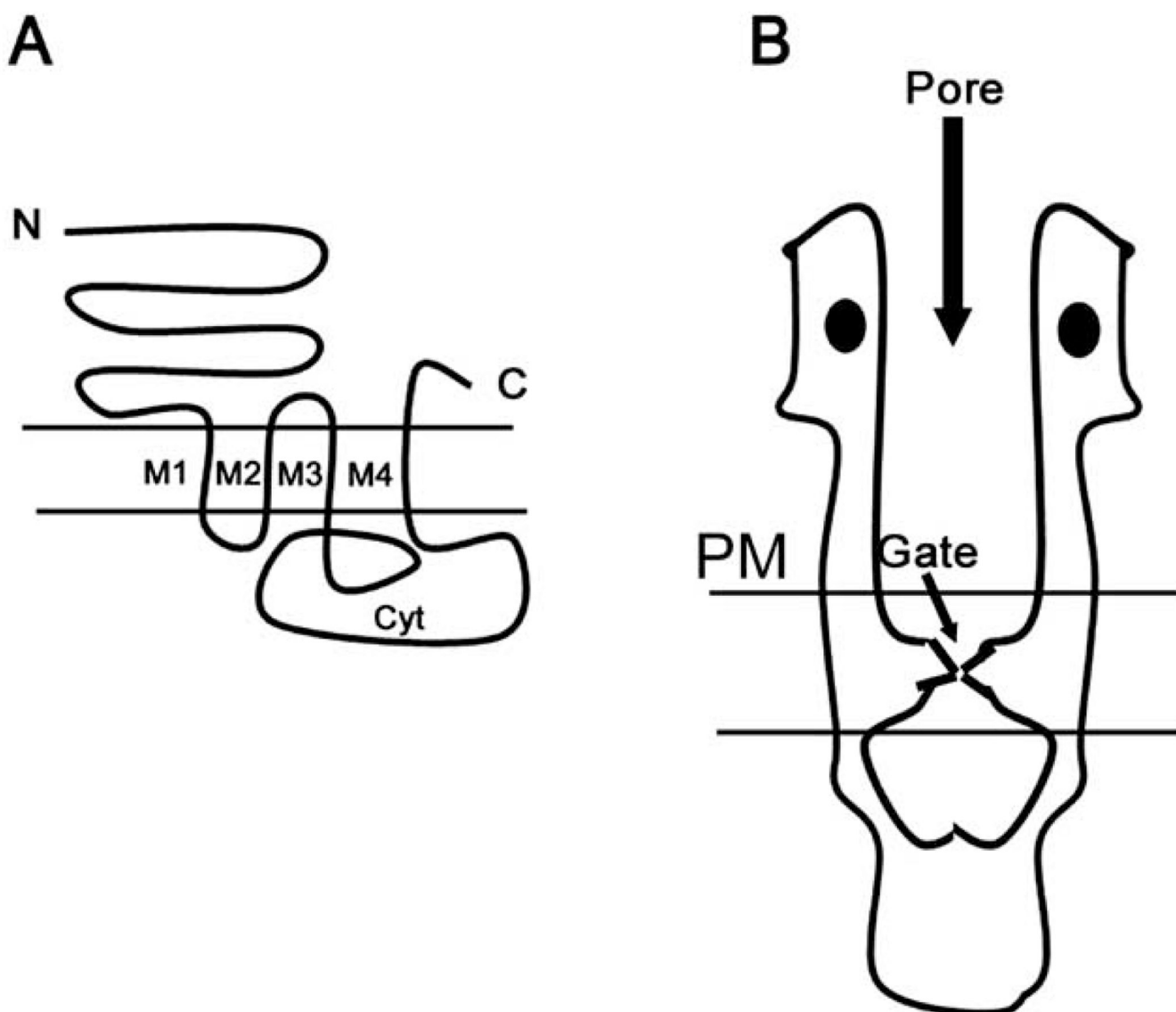


Fig. (3). Basic Structure of nAChRs

A. The figure shows a typical 4 transmembrane topology of nAChRs from the N- to C- termini (N & C). Transmembrane domains are denoted (M1–M4). There is a large cytoplasmic loop separating M4 from M3 (cyt).

B. A low resolution model of nAChRs based on cryo-EM and X-ray crystallographic studies. There is a large N-terminal portion of the protein that forms the extracellular portion of the receptor. The distance between residues lining the ion channel pore opening narrows as the protein spans the plasma membrane. The narrowest portion of the pore lining region, lined by a ring of leucine residues, forms the channel gate. This is followed by the large cytoplasmic region. The two ligand-binding domains are depicted as filled ovals in the figure.

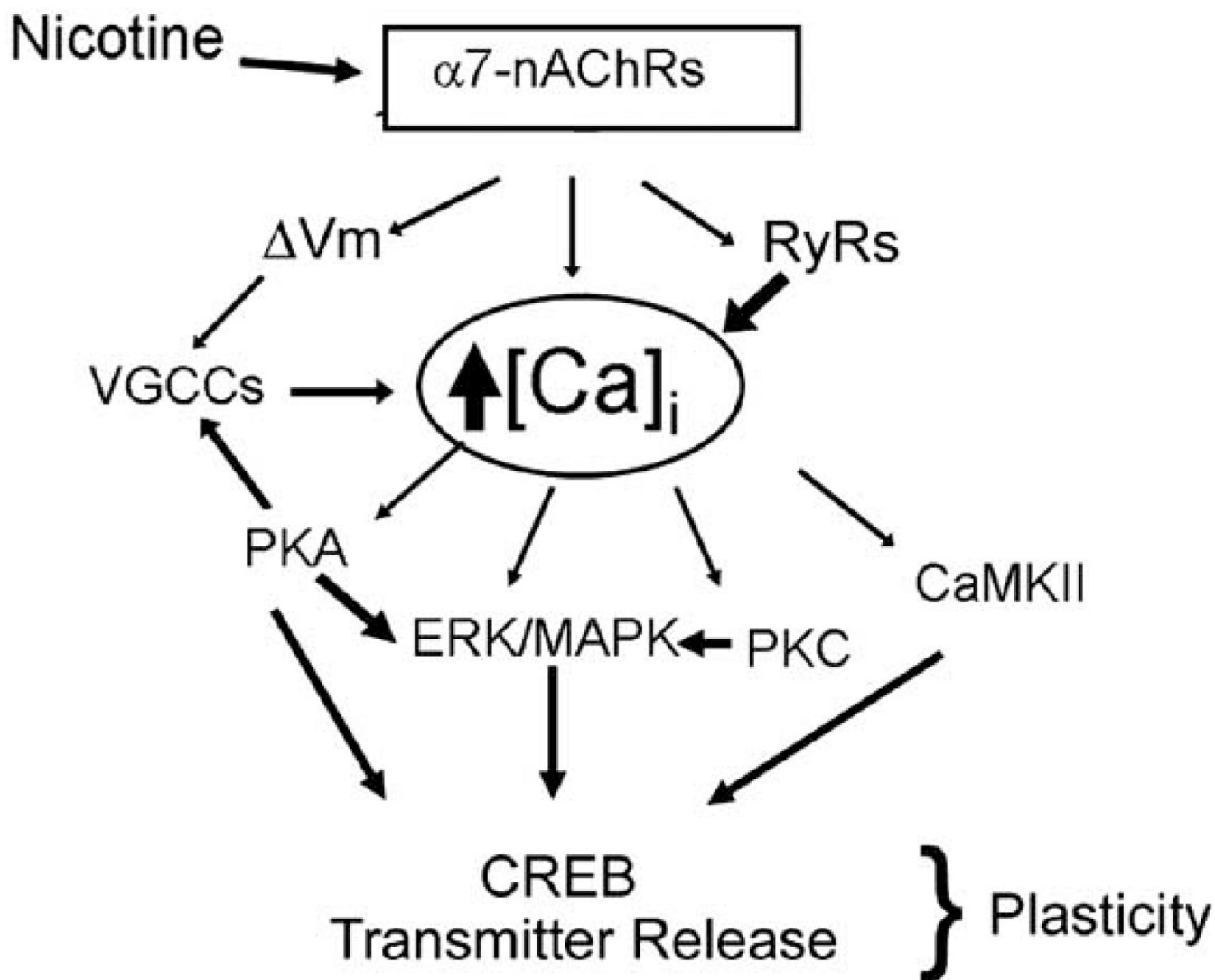


Fig. (4). α7-nAChRs and calcium-dependent signal transduction

Activation of α7-nAChRs by nicotine, triggers an efficient calcium-dependent signal transduction cascade. Calcium flux through upon activation of α7-nAChRs by nicotine is amplified downstream by membrane depolarization (ΔV_m) and activation of VGCCs as well as by CICR from the endoplasmic reticulum via RyRs. The rise in intracellular free calcium levels results in activation of multiple calcium-dependent kinases resulting in short- and long-term changes in synaptic plasticity.