From ligand design to therapeutic efficacy: the challenge for nicotinic receptor research

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***S*-Nicotine, the principal psychoactive constituent of *Nicotiana tabacum*, underpins addiction to tobacco smoking. Although tobacco consumption is a leading cause of death worldwide, nicotine itself is also proposed to have potential therapeutic benefits for a diverse range of conditions. Nicotine interacts with its cognate receptors in the central nervous system to exert a predominantly modulatory influence, making neuronal nicotinic receptors attractive therapeutic targets. Here, we focus on three natural products as lead compounds for drug discovery programs, nicotine, epibatidine and cytisine, and consider the aims and limitations that shape these drug discovery endeavors.**

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Heterogeneity of nicotinic acetylcholine receptor: which subtype to target?

Nicotine (Figure 1) is the classical agonist of a family of ligand-gated cation channels that also respond to acetylcholine (ACh, Figure 2) as the endogenous ligand, hence defined as nicotinic acetylcholine receptors (nAChRs). These pentameric receptors are generated from different sets of subunits expressed in mammalian skeletal muscle ($\alpha1$, $\beta1$, γ, δ, ε), neurons ($\alpha2$–$\alpha7$, $\beta2$–$\beta4$) and sensory epithelia ($\alpha9$, $\alpha10$). In autonomic neurons, nAChRs responsible for synaptic transmission comprise $\alpha3$ and $\beta4$ subunits, with the possible addition of $\alpha5$ and/or $\beta2$ subunits (hence $\alpha3\beta4$*, where * indicates the presence of one or more additional types of subunit [1]). $\alpha3\beta4$* nAChRs are also expressed in the central nervous system (CNS) to a limited extent, whereas the $\alpha4$ subunit is exclusively expressed in the CNS. In combination with the $\beta2$ subunit, $\alpha4\beta2$* nAChRs are the most abundant and widespread nAChR subtypes in the brain, exhibiting high affinity for nicotine. Homomeric $\alpha7$ nAChRs are also prevalent and are distinguished by their high relative permeability to Ca^{2+} [2]. In contrast to the well-defined roles of nAChRs in mediating synaptic transmission at muscle end plates and in autonomic ganglia, neuronal nAChRs in the brain have rarely been shown to fulfill this function, instead being found on presynaptic terminals or at extrasynaptic locations on soma and dendrites. Together with their capacity for interfacing with cellular signaling pathways, this has generated the view that neuronal nAChRs exert a predominantly modulatory influence in the CNS [3].

A modulatory action offers attractive therapeutic opportunities and interest has focused on emulating nicotine's effects by generating agonist molecules. However, nicotine activates all subtypes of nAChRs (except those comprising $\alpha9$ and $\alpha10$ subunits [4]), albeit with different potencies. Issues for drug discovery include nAChR subtype selectivity and addiction liability. In addition to generating agonists with low potency at muscle and ganglionic nAChRs (to minimize peripheral side effects), is it advantageous to target specific neuronal nAChR subtypes (e.g. $\alpha7$ versus

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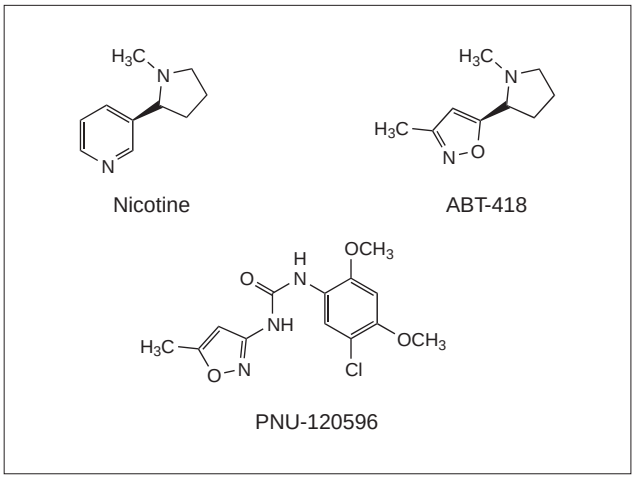

FIGURE 1

Chemical structures of nicotine, its isoxazole analogue ABT-418 and α7-specific potentiator PNU-120596. Reversing the heteroatom positions in the isoxazole ring and introducing a hydrophobic extension of the basic sidechain changes an agonist into a positive allosteric modulator.

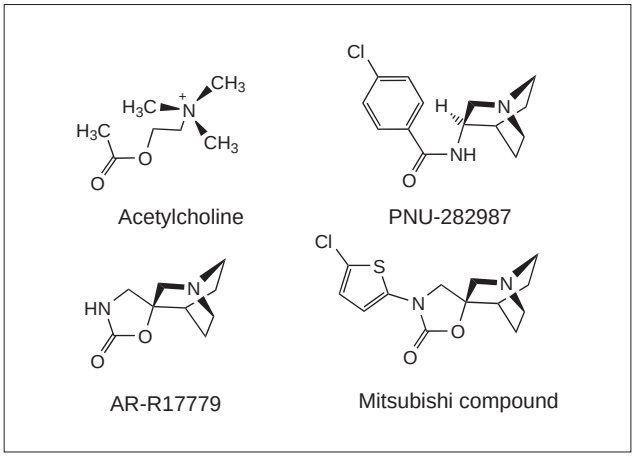

FIGURE 2

The acetylcholine pharmacophore, held in a rigid scaffold, produces selective α7 nAChR agonists.

α4β2*)? Although the answer will differ for different clinical scenarios, some studies have implied that α7 and β2* subtypes conspire together to effect neuroprotection [5,6], in which case selectivity would be inappropriate unless combination therapy with complementary subtype-selective drugs could be achieved. And which nAChR subtypes to target? To date, most effort has focused on targeting the most prevalent subtypes, α7 or α4β2 nAChRs [7]. Less abundant subtypes with more restricted patterns of localization could offer more-selective therapeutic targets, but the potential for nAChR heterogeneity arising from different subunit combinations, with subtle differences in properties or regulation, far exceeds our current knowledge of native nAChR subtypes, making informed decisions about more-complex combinations difficult or impossible at present. Addiction liability is probably not a major concern, unless novel compounds are likely to be smoked: cigarettes provide the optimum delivery device for nicotine self-administration, accompanied by plenty of associated cues and Pavlovian reinforcement; nicotine replacement therapies demonstrate the low liability associated with alternative routes of delivery.

Agonist, antagonist or partial agonist?

To achieve clinical efficacy, is activation or inhibition of nicotinic signaling required? This is a challenging question and the answer is likely to depend on the particular nicotinic contribution to each clinical target. Nicotine, the prototype with credentials for improving several conditions, is regarded as an agonist of muscle and neuronal nAChRs but it is also a powerful desensitizing ligand that can produce functional deactivation of nAChR after a few seconds or minutes of exposure (depending on nAChR subtype and nicotine concentration) [8]. Functionally, nAChR can exist in four distinct conformations: resting, open and two desensitized states. The latter are refractory to activation on a time scale of milliseconds or minutes depending on the desensitized state but have high affinity (pM–nM) for agonists. Chronic nicotine exposure can induce a long-lasting functional deactivation as a result of rapid and persistent desensitization. Clinical prescription of drugs will involve chronic administration and the interplay of activation and desensitization is likely to be crucial, as well as the contribution of potentially bioactive metabolites. At present, modeling this relationship for human brain nAChRs is simplistic, even for nicotine in relation to cigarette smoking [9], and impossible to predict for novel ligands. However, because cognitive enhancing and neuroprotective effects of nicotine are abolished by coadministration of nAChR antagonists [5,6,10], activation of nAChRs is generally sought and attention has focused on generating small agonist ligands. Tourette's syndrome, on the other hand, might present a case for nAChR antagonists (see below).

Partial agonists provide a compromise solution that could have particular utility, for example, for smoking cessation. In this regard, cytisine was noted for its selective partial agonism at α4β2* nAChR: this subtype plays a major role in governing mesolimbic dopamine release in response to nicotine, which underlies the reinforcing properties of the drug. In fact, cytisine (Tabex®) is marketed in Eastern Europe as an aid for smokers wishing to break their habit. It has been argued that binding of a partial agonist at this receptor would provide a modicum of dopamine release to compensate for the absence of nicotine (providing some relief from withdrawal symptoms), whereas occupancy of the nAChR prevents nicotine from binding [11,12]. The molecular interactions that result in partial agonism are not understood and it is problematic to predict the activity of any derivative.

Although beyond the remit of this review, it is worth mentioning the current interest in another class of nicotinic ligand, nAChR potentiators [13]. Such molecules produce no obvious nicotinic response alone but enhance

responses to conventional agonists (including endogenous ACh). The Alzheimer's disease (AD) drug galanthamine is credited with allosteric potentiation of nicotinic responses, in addition to its anti-cholinesterase activity [13,14]. Whereas galanthamine does not discriminate between nAChR subtypes, an α7-specific potentiator PNU-120596 (Figure 1) has recently been described [15]. A search for a subtype-selective negative modulator, to depress nAChR responses, has been advocated for treating familial epilepsy [16] (see below).

Clinical targets for nicotinic drugs

The development of potential nicotinic therapeutics was prompted initially by the effects of nicotine taken in the form of tobacco products. Epidemiological evidence shows a negative correlation between smoking and the incidence of Parkinson's disease (PD) and, to a lesser extent, AD [17]. Patients with schizophrenia or other mental illnesses and adolescents with attention-deficit hyperactivity disorder (ADHD) show increased tobacco consumption, equated with attempted self-medication [18], whereas, anecdotally, smoking improves Tourette's syndrome and ulcerative colitis and quitting smoking worsens these conditions. Such observations, together with animal studies, have identified several diverse clinical conditions that might benefit from new nicotinic drugs.

Cognitive performance

Analysis of the ability of nAChR stimulation to improve cognitive function has identified attentional performance as the most likely component to be positively influenced by nAChR activation. These effects are more readily seen in individuals with pathological disease states, rather than normal individuals [18]. In animal models, nicotinic agonists improve learning, memory [19] and attention [20]. Current targets for nicotinic drug development for improved cognitive performance include mild cognitive impairment, schizophrenia, ADHD, AD and PD [18].

Neurodegenerative diseases

An involvement of nAChRs in AD and PD is also supported by the consistent loss of nAChRs in post-mortem brain tissue from patients, which, in the case of AD, significantly correlates with cognitive impairment [21]. Clinical trials of nicotine or nicotinic agonists in AD patients have reported improvement in attentional capacity and some verbal and nonverbal skills and encourage the development of more-selective nicotinic drugs for use in AD [18].

The case for nicotinic therapy in PD rests on the ability of nicotine and other agonists to evoke dopamine release, in addition to exerting a putative neuroprotective effect on neurons [22]. Clinical studies assessing the efficacy of nicotine in PD have generated inconsistent results. Although some studies demonstrate that smoking alleviates some PD symptoms, others using transdermal nicotine treatment show no improvement or adverse effects [22,23]. The latter appear to reflect autonomic stimulation and emphasize the need for better subtype-selective nAChR agonists. The restricted localization of α6* nAChRs to catecholamine systems in the brain, where they enhance transmitter release, might make this subtype a credible target for PD, if selective agonists could be found [22].

Schizophrenia

α7 nAChRs are reduced in number in the post-mortem brains of schizophrenic patients and nicotine is beneficial in transiently normalizing the sensory gating deficit, as well as improving some cognitive deficits [24,18]. The association of the α7 nAChR subunit gene with the sensory gating deficit [25] has provoked interest in α7-selective agonists for treating schizophrenia.

Anxiety and depression

Nicotine can modulate the neurotransmitter systems involved in stress responses, anxiety and depression in the normal brain, notably monoamine systems [26]. However, nicotine can be either anxiolytic or anxiogenic, depending on factors such as the anxiety model tested, the route of nicotine administration, dose and time course of administration; heterogeneity of nAChR subtypes might underlie some of the paradoxical effects of nicotine. Clinically, nicotine has been reported to improve compulsive aspects of obsessive–compulsive disorder in humans and in animal models [27].

Tourette's syndrome

This condition involves uncontrolled obsessive–compulsive behavior, as well as motor (especially facial) and vocal tics. Transdermal nicotine reduces the behavioral symptoms and can offer additional benefits, if given in conjunction with haloperidol [28]. The hypothesis to account for the beneficial effects of nicotine is that it desensitizes nAChRs present on dopaminergic neurons, thereby limiting dopamine release. Hence, nicotine is effectively acting as an antagonist, and this is supported by the similar efficacy of the antagonist mecamylamine in treating Tourette's syndrome [29].

Epilepsy

Mutations in the channel-forming domain of α4 or β2 subunits give rise to a rare epilepsy syndrome: autosomal dominant nocturnal frontal lobe epilepsy [16]. At a molecular level, the mutations increase sensitivity to ACh. This condition presents a case for a negative allosteric modulator of nAChR to reduce the impact of excessive agonist activation [16].

Pain

One of the most promising therapeutic applications of nicotinic agonists is in the control of pain. The antinociceptive activity of nicotine was described 70 years ago but, because of its short-lived effect, it has been overlooked,

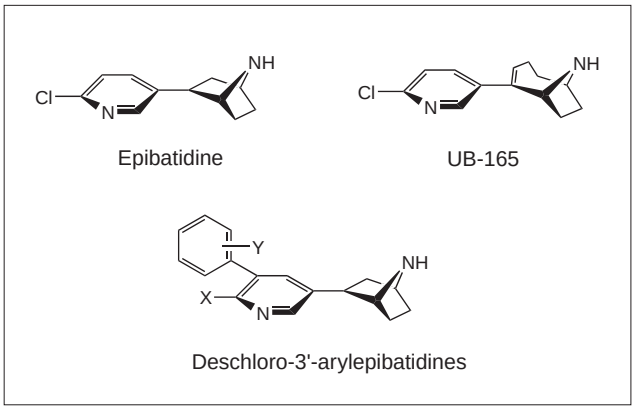

FIGURE 3
Chemical structures of epibatidine and synthetic analogues.

and the development of nicotinic agonists as analgesics started only after the description of the antinociceptive effects of epibatidine (Figure 3), which exceed those of morphine [30]. Epibatidine's antinociceptive activity is compromised by serious side effects at similar doses, reflecting its relatively nonselective interaction with nAChR subtypes; analgesia is attributed to α4β2* nAChR but other subtypes could also contribute [31,32].

Smoking cessation

Combating nicotine addiction represents the most tangible case for a nicotinic therapy. Nicotine itself is available in various delivery systems for nicotine replacement therapy: although this strategy doubles the success rate of smokers attempting to quit the habit, approximately three out of four have failed to give up smoking when assessed one year later. The antagonist mecamylamine can also ameliorate some nicotine-induced effects in smokers in the clinic but results in increased craving [33]; a partial agonist approach has recently been advocated [11,12]. Intriguingly, nicotinic mechanisms might also contribute to the self-administration of other drugs, including alcohol and cocaine [33], but the therapeutic potential of nicotinic drugs for treating other addictions has not been explored.

Ulcerative colitis

This chronic relapsing inflammatory bowel disease is largely restricted to non- or ex-smokers. Consequently, transdermal nicotine [34] and nicotine enemas [35] have been tested and found to improve the symptoms. The underlying mechanism is assumed to involve nicotinic stimulation of parasympathetic innervation or possibly modulation of local inflammatory responses [36]. However, the adverse effects associated with nicotine, especially with transdermal delivery, encourage the development of better nicotinic drugs to combat this disease. The recent finding that α7 and α4β2 nAChRs regulate B-lymphocyte activation and immune responses suggests a possible new therapeutic approach to a variety of other inflammatory conditions [37].

Ligand evaluation: the challenge for drug screening models

The diversity of nAChRs presents a challenge for the development of relevant high throughput screens to ascertain drug selectivity. This is exacerbated by pharmacological differences between nAChR homologues of different species [38,39], despite a high sequence homology across species for individual subunits [40]. Heterologous expression of human recombinant nAChRs has addressed the target species but is compromised by our limited knowledge of the subunit composition of native human nAChRs. However, the expression of functional nAChRs with defined subunit combinations has contributed to the identification of putative native subunit combinations and potential therapeutic targets, and to this end provides the most practical tool for studying the pharmacological and functional properties of identified nAChR. To the first approximation, pharmacological and functional properties of heterologous and native nAChR are similar [41–44], although biophysical properties can be affected by expression of human nAChR in *Xenopus* oocytes [44]. An important limitation of current transient expression systems is the difficulty in obtaining homogeneous populations of heteromeric nAChRs, particularly of receptors containing more than two different subunits (e.g. α6β4β3α5 [45]). Furthermore, current stable or transient expression technologies do not allow the expression of a homogeneous nAChR population in which only one of several otherwise identical subunits is mutated, as in many dominantly inherited channelopathies [16].

In addition to the application of heterologously expressed nAChRs for ligand binding and electrophysiological assessment of novel ligands, downstream functional readouts (e.g. changes in intracellular Ca^{2+}) can be used [46]. Native nAChRs expressed in neuroblastoma cell lines (e.g. human SH-SY5Y cells) offer an alternative to heterologous expression but such preparations represent autonomic (rather than CNS) neurons. nAChR-mediated transmitter release from brain slices has been adapted for a 96 well format, to provide an assay of native (albeit rodent) nAChRs: this is a complex model, as several nAChR subtypes can regulate transmitter release in each brain area [47].

The interpretation of biochemical and electrophysiological results, as well as the design of novel ligands, is expected to rely increasingly on the computational modeling of nAChRs (See Box 1).

Templates for nicotinic drug design: three natural products

Hereafter we focus on three natural products that have provided templates for many of the nicotinic drug discovery programs. For a comprehensive review of nicotinic ligands the reader is referred to Jensen *et al.* [7].

Nicotine

Nicotine (Figure 1) was in the 1990s the most obvious choice as a lead for the development of nicotinic receptor

BOX 1

Predictive *in silico* modeling

As a complement to 'wet' screening assays, recent progress in the use of *in silico* modeling has followed publication of the crystal structure of the HEPES-bound *Lymnaea stagnalis* acetylcholine binding protein (AChBP). This novel protein shares sequence similarity with the N-terminal, extracellular domain of nAChR subunits, particularly α7, and is also a homomeric pentamer [80]. Using the AChBP structure as a template, several 3D models of the ligand-binding domains of nAChR subtypes with ACh, nicotine, epibatidine and cytisine docked to the binding site have been published [81–84]. Estimates of the free energy of binding of acetylcholine, nicotine and epibatidine reproduced the rank order of experimentally determined values for these ligands [82], and the DOCK scores of ACh, nicotine and cytisine indicated an order (cytisine > nicotine = ACh) in agreement with experimental results [83]. Docking of cytisine, *N*-methylcytisine and several of their halogenated derivatives to models of various human neuronal nAChR subtypes also reproduced the proper order of affinities, suggesting that these models are reliable tools to interpret experimental results and might be useful for prediction of affinities of new compounds [G. Zapata-Torres, personal communication].

An important limitation of such modeling studies is the absence of the membrane-spanning helices and intracellular domain of nAChR, which, if not crucial for agonist binding to a static receptor conformation, are expected to play an important role in receptor dynamics. Thus, although models based on the AChBP structure reproduce agonist binding (and affinities) reasonably well, they cannot provide good estimates of ligand efficacies. As clearly indicated by Celie *et al.* [78], no conformational changes were observed in the AChBP that could explain receptor gating. However, a most recent development that will hopefully help to overcome this drawback has been the use of the X-ray crystal structure of the snail AChBP [80] together with more recent cryo-electron microscopy data of the membrane domains of Torpedo nAChRs [85] to build a model of the α7 nAChR to explore its gating mechanism [86].

agonists. SARs at α4β2, and in some cases α7, nAChR of ligands known until the year 2000 have been thoroughly reviewed by Schmitt [48]. Pyrrolidine ring *N*-substitution with groups larger than ethyl is not tolerated and any substituent other than methyl, monofluoromethyl or ethyl on the pyrrolidine carbon atoms leads to losses of α4β2 affinity of more than one order of magnitude. Ring expansion to the natural anabasine or its *N*-methyl analogue results in an important loss of affinity, whereas contraction to the azetidine analogue causes a slight increase. However, replacement of the pyrrolidine ring by bicyclic amines leads in some cases to compounds with similar affinities to nicotine and, in the case of the 7-azabicyclo[2.2.1]heptane system of epibatidine, to analogues with much greater affinity. Introduction of a fluorine, chlorine or bromine atom at the C-6′ position, congruent with the halogenated carbon of epibatidine, affords compounds with slightly (two- to five-fold) enhanced α4β2 affinity, and a methyl or ethyl group at the same position is well tolerated but substitutions at any other position on the pyridine ring are unfavorable. A properly oriented isoxazole ring can replace the pyridine ring with little loss of affinity relative to nicotine, as in ABT-418 (Figure 1). Two series of ethano-bridged nicotine analogues exhibit rather poor binding affinities.

These generally disappointing results might explain why many less direct offshoots with various spacers between the aromatic heterocycle and the more basic nitrogen atom, such as the 3-pyridinyl and other heteroaryl ethers and thioethers, as well as heteroarylmethylene azacyclic compounds, have been synthesized and assayed. Structures derived from a rigid acetylcholine (or more exactly, carbamoylcholine) analogue, such as AR-R17779, PNU-282987 [49] and a related compound published by a group at Mitsubishi Pharma Corporation [50] are purported to be selective α7 nAChR agonists (Figure 2).

Epibatidine

Ever since the potent analgesic properties of epibatidine (Figure 3) were linked to its ability to activate nicotinic receptors [51–53] and the development of appropriate synthetic methodologies [54,55], structural modifications have been carried out as prerequisites to understand the basis of the exceptional potency of epibatidine and to explore the possibility of finding even more potent or more selective compounds. The 5-(2-chloropyridinyl) ether ABT-594, which achieves an epibatidine-like distance between the pyridine and the more-basic azetidine nitrogens, was abandoned after completing Phase II clinical tests as an analgesic because of its gastrointestinal side effects (http://media.corporate-ir.net/media_files/nys/abt/rdday/NeuroPain.pdf). The pyridyl ether 3(2*S*-azetidinylmethoxy)pyridine (A-85380) was developed to retain the potency of epibatidine while gaining greater selectivity for central nAChR [56]. This compound and its 5-iodo analogue, developed for *in vivo* single-photon emission tomography (SPECT) imaging, have been useful for distinguishing subpopulations of nAChR binding sites in mammalian brain, including selective decreases in striata from PD patients [57].

The SARs of epibatidine and its analogues have been reviewed recently [58], mainly with regard to nAChR affinity and antinociceptive activity, and earlier work will not be discussed in detail. A small number of structural analogues, *N*-substituted, with different pyridine ring substituents, with bioisosteric substitution of the pyridine ring for other aromatic heterocycles, with changes in the azabicyclic system or with conformational restraints, are nearly equipotent with epibatidine. However, most epibatidine analogues have lower (usually much lower) affinities and are not distinguished for their subtype selectivity (although this has not been extensively examined). A recent exception is a 5-alkynyldeschloroepibatidine which has half the affinity of epibatidine at α4β2 nAChR but is more than 2000-fold selective with regard to the α4β4 subtype [59]. In the cases in which functional assays have

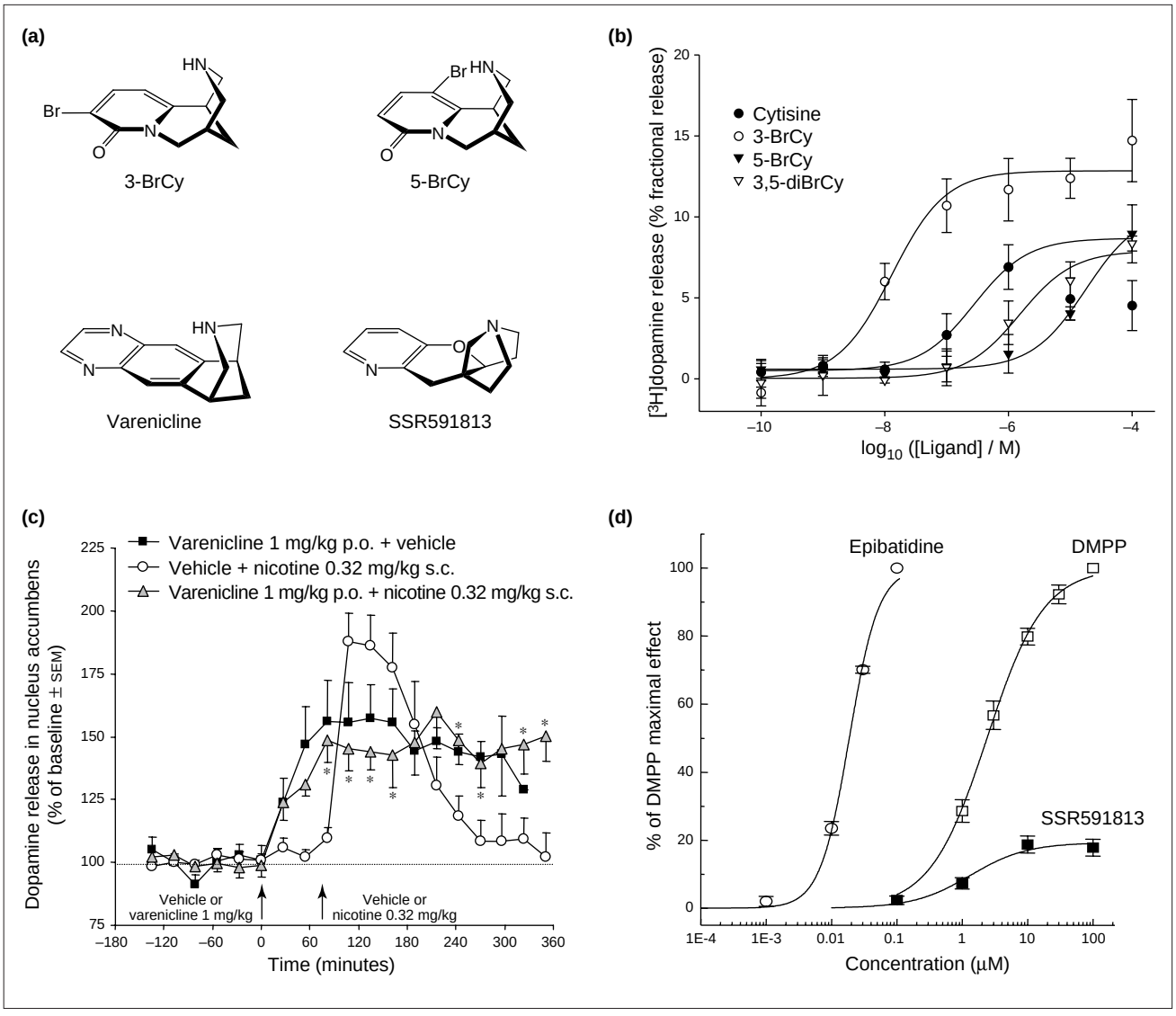

FIGURE 4

Cytisinoids exhibit a wide range of potencies and efficacies. (a) Chemical structures of 3-BrCy, 5-BrCy, varenicline [12] and SSR591813 [11]. **(b)** nAChR-evoked [^{3}H]dopamine release from rat striatal slices *in vitro*. In this assay, governed mainly by α4β2* nAChR, cytisine is a partial agonist, whereas 3-BrCy is more potent and more efficacious. 5-BrCy and 3,5-BrCy have diminished activity compared with cytisine (Abin *et al.*, personal communication). **(c)** Time courses for the effects of nicotine (0.32 mg/kg s.c.; open circles) and a maximally effective dose of varenicline (1.0 mg/kg p.o.) alone (filled squares) and in combination with nicotine (triangles) on extracellular dopamine levels in the nucleus accumbens of conscious Sprague-Dawley rats. Varenicline was administered 1 h before nicotine (arrows), and effects on dopamine release are expressed as a percentage of baseline (mean of last 5 predrug basal levels) ± SEM (n = 4–6). *p<0.05: varenicline with nicotine versus nicotine alone (2-factor analysis, repeated measures, Western-Electric). Varenicline alone is less efficacious than nicotine, but it also reduces the response to nicotine, consistent with a partial agonist interaction with the nAChRs mediating this response. Part (c) reproduced, with permission, from Ref. [12]. **(d)** Effects of SSR591813 at human α4β2 nAChRs expressed in *Xenopus* oocytes. Concentration response curves for epibatidine, DMPP and SSR591813 show that SSR591813 has low efficacy relative to the other agonists with respect to evoking whole cell responses. For each agonist, data points indicate the mean ± SEM of current amplitudes (n = 2–6 oocytes), normalized to 100 μM DMPP. Part (d) reproduced, with permission, from Ref. [11]. Abbreviations: 3-BrCy, 3-bromocytisine; 5-BrCy, 5-bromocytisine; s.c., subcutaneous; p.o., *per os* (orally).

been performed, these compounds are less potent than epibatidine. Unexpectedly, UB-165 (Figure 3), a synthetic hybrid of the efficacious α4β2* nAChR agonists epibatidine and anatoxin-a, is a partial agonist at this subtype [60].

Recently, a series of deschloroepibatidine analogues bearing halogen or other small substituents at C-3′ were characterized [61]. These compounds resemble C-3′-arylepibatidines and deschloro-2′-substituted epibatidines (Figure 3) [62,63] in their high affinity for α4β2 nAChR and weak or negligible affinity for α7 nAChR, combined with weak agonist or antagonist activities in pain, body temperature or spontaneous activity tests. The high affinity (K_i = 0.029 nM versus [^{3}H]epibatidine in rat brain cerebral cortical membranes), weak agonist and potent antagonist activity of *N*-methyl-3′-iododeschloroepibatidine have led to the suggestion that its ^{11}C and ^{125}I analogues might be useful as ligands for *in vivo* studies of nAChR using positron emission tomography and SPECT, respectively [61].

Cytisine

Although cytisine was recognized as a nicotinic agonist as far back as 1912 [64] and its affinity for neuronal nAChR is greater than that of nicotine [65], its structure has only been exploited very recently as a template for the synthesis of new and novel compounds for testing as possible nicotinic drugs (Figure 4).

Systemic administration of cytisine typically affects the autonomic ganglia, the adrenal medulla and the CNS [66]. Regardless of possible changes in affinity and efficacy at nonganglionic nAChR subtypes, increasing the lipophilicity of the very hydrophilic cytisine [67] would be expected to improve blood–brain barrier penetration, thus heightening the effects of its derivatives in the brain. Nevertheless, *N*-methylation not only reduced its potency in ganglionic and striated muscle preparations [68], but also decreased its affinity and functional potency *in vitro* at human α7, α4β2 and α4β4 nAChRs [69].

Another way of increasing overall lipophilicity is by introducing hydrophobic groups on the α-pyridone ring, which is easily accomplished as a result of the partially aromatic character of this moiety. A large number of derivatives were prepared in this way *en route* to possible positron-emitting radioligands [70], but no biological results were reported by these authors. A year later, however, the first reports appeared on the affinities and functional properties of several pyridone ring-halogenated derivatives [71,72] (Figure 4), which were supplemented shortly with new results including two bromo-derivatives of *N*-methylcytisine, also known as caulophylline [69] and *N,N*-dimethylcytisinium or caulophylline methiodide [73]. A generalization that emerges is that halogen substitution at C-3 (also designated in some papers as C-9) always leads to increased affinity and functional potency, whereas halogenation at C-5 (or C-11) results in modest decreases and dihalogenation leads in some cases to further loss of activity [69] (Abin-Carriquiry *et al.*, personal communication). All the cytisine derivatives tested have much lower affinity and potency at homomeric α7 nAChR than at the α4 subunit-containing subtypes, as is also the case for the parent compound.

In addition, the thio analogue of cytisine with a sulfur atom in place of the pyridone oxygen was prepared and tested for affinity in rat forebrain membranes [71] and later in cells expressing rat α3β4 or human α4β2 and neuromuscular nAChR [73]. This structural modification resulted in several-fold reductions in affinity and functional potency toward α4β2 and neuromuscular nAChR but much greater decreases at brain α7 nAChR and at α3β4 receptors. However, the most structurally distant cytisine congener, with a symmetrical structure derived from 3-substituted cytisinoids, is the potent α4β2 partial agonist varenicline (Figure 4), which has potential medicinal utility with respect to dopamine release and has entered clinical trials as a possible treatment for tobacco dependence [12]. Another partial agonist with similar topology is SSR591813 (Figure 4), also proposed as an aid for smoking cessation [11].

Common features and differences

A common feature of nicotine, epibatidine, cytisine, and their analogues is their low affinity for α7 nAChR relative to α4β2 nAChR. 3D QSAR analyses have not added much to our understanding of the structural requirements for potent nicotinic agonist activity, regardless of the methodology used, beyond confirming the relevance of a properly positioned positive charge and a hydrogen bond acceptor [74]. Recently, a study using comparative molecular field analysis and comparative molecular similarity analysis on a large set of epibatidine analogues showed that steric and electrostatic interactions in the area near the chlorine atom of epibatidine are favorable features, suggesting in addition that the volume contributed by the saturated bicyclic system of epibatidine also enhances affinity, possibly by ensuring an appropriate orientation of the charged nitrogen atom [75]. Similar conclusions were reached using distance comparison, quantitative structure-affinity relationships and models generated by multi-objective genetic QSAR [76].

Whereas nicotine has 70-fold lower agonist potency than ACh at the neuromuscular nAChR, epibatidine is a potent agonist at neuromuscular and neuronal nAChR. This difference has been rationalized within the framework of 'cholinergic' and 'nicotinic' receptors by measuring the binding affinities of ACh, nicotine and epibatidine to nAChR mutated with unnatural amino acids [77]. These authors conclude that the high potency of epibatidine results in part from its binding to the active site Trpα149 residue via a strong cation-π interaction and a hydrogen bond to the backbone carbonyl oxygen atom. The quaternary cationic center of ACh is unable to form a conventional hydrogen bond and nicotine does not form a strong cation-π interaction [77]. Although the distance separating the charged nitrogen atom of protonated nicotine from the centroid of the Trpα149 benzene ring [78] is similar to the corresponding distances calculated for (+)- and (–)-epibatidine [76], the cation-π interaction in epibatidine is expected to be stronger because of the more-positive electrostatic potential of its cationic centre as compared with that of nicotine [79]. The binding of epibatidine (and to a lesser extent of nicotine) might be reinforced further by a nonclassical hydrogen bond (C–H···O=C) involving the hydrogen atom at C-2 of the pyridine ring, which is expected to bear a greater partial positive charge in epibatidine than in nicotine as a consequence of the electron-attracting character of the chlorine atom [76].

The different effects of substitution on the pyridine ring of nicotine and epibatidine analogues, on one hand, and of the α-pyridone ring of cytisinoids, on the other hand, remain unexplained. Thus, the strong increases in affinity for neuronal nAChR of cytisine when brominated or iodinated at C-3 [69] and of varenicline, which might be regarded as a 3-substituted, ring-contracted cytisinoid isostere [12], have little parallel in the nicotine and epibatidine

series. Introduction of a halogen atom (F, Cl, or Br) at the C-6 position of nicotine, corresponding to the C-2′ position of epibatidine and neighboring the putative hydrogen bond accepting site, as is the case for the C-3 position of cytisine, results in very modest (<threefold) increases in the displacement of [^{3}H]nicotine from rat brain membrane binding sites, and a wide variety of other groups have a deleterious effect [76]. Removal of the C-2′ chlorine atom of (+)- or (–)-epibatidine and its replacement by fluorine, bromine or iodine has almost no effect on their affinities [58]. The functional potencies of these nicotine, epibatidine and cytisine analogues have been assayed in widely differing models and are not easily comparable. Nevertheless, the available results also point to varying trends suggestive of different binding modes or mechanisms of activation. Another striking difference is the relative insensitivity of nicotine and epibatidine to the presence or absence of an *N*-methyl group, whereas *N*-methylation of cytisine leads to sixfold, 25-fold and 300-fold losses of affinity for α4β2, α7 and α4β4 nAChR, respectively [69]. The fact that nicotine enantiomers differ by a factor of 30 in their affinities for rat brain high affinity nAChR binding sites, whereas (+)- and (–)-epibatidine are almost indistinguishable [58], is also rather surprising. However, the latter observations should be regarded with caution, as the behavior of some of these compounds toward individual receptor subtypes does not seem to have been assessed.

Conclusions

Extensive structure–activity studies taking natural nicotinic agonists as the starting point have begun to reveal some rules for enhancing agonist potency, although these rules are often not intuitive and improvements in nAChR subtype selectivity have been more elusive. To date, few novel nicotinic compounds have succeeded in clinical trials, emphasizing the limitations in translation from screening models to clinical application. However, by coupling structure–activity studies with recent advances in the modeling of ligand interactions with nAChR binding sites, the future looks promising for more-successful predictive drug design to generate new nicotinic drugs for the diverse conditions where they could offer therapeutic benefits.

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