Nicotine: not just for cigarettes anymore

Only a few years ago, the only interest in the actions of nicotine on the brain was with respect to its psychoactive and addictive properties. Any involvement of nicotinic receptors with synaptic transmission was viewed with scepticism. Subsequently, their existence and diversity in the brain was confirmed by the cloning and characterization of genes encoding nicotinic subunits from vertebrate (chicken, rat, human) neurones. In various combinations, these form pentameric, ligand-gated cation channels that can be activated by acetylcholine, nicotine and related molecules (hence, nicotinic acetylcholine receptor, nAChR) [Role, L.W. and Berg, D.K. (1996) *Neuron* 16, 1077–1085]. These discoveries have provided the stimulus to understand the molecular structure, function and physiological significance of neuronal nAChR subtypes. In turn, these studies have generated interest in nAChR as therapeutic targets for diverse neurological and psychiatric conditions. Numerous pharmaceutical and biotechnology companies worldwide now have drug discovery programmes directed at the development of nicotinic ligands. It was the recognition of these exciting times in nAChR research that prompted the IBC conference on *Nicotinic acetylcholine receptors: advances in molecular pharmacology and drug development* in Annapolis (MD, USA) on 13–14 May 1999.

Potential clinical applications for nicotinic drugs

The perception of nicotine as being 'bad', from its association with tobacco and its reinforcing properties that sustain tobacco use, has evolved to that of being 'good' (or at least 'not quite so bad'). This change has followed its use clinically for the treatment of Tourette's syndrome and ulcerative colitis (discussed by William Sandborn, Mayo Medical School, Rochester, MN, USA), as well as its application as a smoking cessation agent. Furthermore, epidemiological studies have suggested that smoking, presumably through the effects of nicotine, provides some protection against the development of Alzheimer's and Parkinson's disease. Tobacco use is prevalent among those suffering from schizophrenia and affective disorders and is reputed to be a form of self-medication. It would be imprudent to advocate smoking as a preventative for such diseases, but over 50 studies report beneficial effects of nicotine on these conditions, as well as on pain management and attention deficit hyperactivity disorder (ADHD). However, nicotine exhibits relatively little selectivity of action, leading to several undesirable side-effects. The development of nicotinic drugs with more subtype selectivity and less dependence liability than nicotine itself has become a goal of the pharmaceutical industry.

Nicotinic receptor subtypes and their properties

One approach to the development of selective nicotinic agents has been to identify and characterize the nAChR subtypes in the brain and periphery, explore their physiology and pharmacology, and identify selective ligands [Holladay, M.W., Dart, M.J. and Lynch, J.K. (1997) *J. Med. Chem.* 40, 4169–4194]. The scope for receptor-subtype diversity is substantial, given that eight α-, four β-, and one each of γ-, δ- and ε-subunits have been identified in mammals. Heterologous expression of nAChR of defined subunit composition has proven useful for determining the profile of responses to classical agonists, antagonists and novel agents.

Laura Chavez-Noriega (SIBIA Neurosciences, La Jolla, CA, USA) discussed the properties of recombinant human neuronal nAChRs expressed in *Xenopus* oocytes or HEK293 cells. While the natural agonist, acetylcholine, has high efficacy for nAChRs comprising α/β-pairs (α2, α3 or α4 with β2 or β4) and for α7-homomers, other agonists (notably cytisine) and antagonists differ significantly in their properties. Agonist affinities are increased by the incorporation of the α5-subunit, giving more complex subunit arrangements. These results reinforce the idea that the development of subtype-selective agonists is achievable.

Extrapolation of results from heterologously expressed subunit combinations to native nAChR requires verification of the subunit composition of the latter. Immunoprecipitation and immunolocalization techniques, and more recently, the availability of null mutant mice lacking particular nAChR subunits has facilitated the allocation of particular subunits to ligand-binding sites, nicotinic responses and behavioural traits, as discussed by Mike Marks (University of Colorado, Boulder, CO, USA).

Clinical implications

nAChR composed of α7-subunits have provoked considerable interest because of their high permeability to Ca^{2+}, their early expression in development, and their anatomical distribution. Darwin Berg (UCSD, CA, USA) presented an

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elegant analysis of the localization and functional significance of α7* nAChR in the calyx synapse of the chick ciliary ganglion, where they are perisynaptic on somatic spines. Functional α7* nAChR appears to be necessary for reliable, synchronized neurotransmission. Edson Albuquerque (University of Maryland, Baltimore, MD, USA) presented evidence that α7* and α4β2 nAChR mediate phasic and tonic responses, respectively, in GABAergic interneurones in mammalian brain. The involvement of both subtypes is consistent with the association of mutations in α7- and α4-subunits with epileptiform activity [Berkovic, S., Curtis, L. and Bertrand, D. (1998) in *Neuronal Nicotinic Receptors, Pharmacology and Therapeutic Opportunities* (Arneric, S.P. and Brioni, J.D., eds), pp. 287–306, Wiley-Liss].

Karen Stevens (University of Colorado, Denver, CO, USA) reported that schizophrenics have decreased numbers of α7* nAChR in their hippocampi and, in some familial cases of schizophrenia, linkage studies map to chromosomal-site 15q14, the location of the α7-subunit gene. α7* nAChR are believed to mediate auditory gating (paired pulse inhibition) that is defective in schizophrenics, and nicotine and the putative α7-selective agonist DMXB (also known as GTS-21) reverses this impairment. DMXB has also been ascribed a neuroprotective function.

While smoking, nicotine and nicotinic agonists demonstrably improve working memory and ADHD (Ed Levin, Duke University, Rahleigh, NC, USA), although the nAChR subtypes responsible have not been unequivocally defined. Drugs acting on the α3β4*- and α4β2-subtypes are in development by SIBIA and Targacept, respectively for Alzheimer's disease, whilst α4β2 nAChR are a plausible target for nicotinic analgesics. Imad Damaj (Virginia Commonwealth University, Richmond, VA, USA) presented some compelling data to support this view, notably the absence or diminution of nicotine's antinociceptive effect in the tail flick and hot plate tests, respectively, in null mutant mice lacking either the α4- or β2-subunit.

However, a potential problem with α4β2-selective drugs is that they might be reinforcing. Chronic nicotine administration results in the development of tolerance and alters both the number and function of nAChR. Cessation of nicotine usage elicits withdrawal. Therefore, the consequences of sustained therapy with nicotinic agents should be considered. As the functional status of increased nAChR numbers elicited by chronic nicotine exposure might depend on the subtype, selective agents will be crucial.

Withdrawal signs, as measured by dysphoria and anhedonia in rats, were described by Athina Markou (Scripps Institute, La Jolla, CA, USA). Cessation of nicotine treatment resulted in long-lasting increases in the threshold for intracranial self-stimulation of the medial forebrain bundle and in the somatic signs of withdrawal. Although the time courses for these two responses were similar, differential effects of nicotinic antagonists suggest that central (anhedonia) and peripheral (somatic) withdrawal effects are dissociated. These observations have implications for smoking cessation strategies that currently emphasize nicotine replacement. Some similarities in withdrawal patterns between opiates and nicotine suggest that partial nicotinic agonists could be useful as cessation products, analogous to partial opiate agonists. Robert Mansbach (Pfizer, Groton, CT, USA) discussed the use of compounds related to cytisine, the well-known partial agonist at β2-containing nAChR. Although cytisine and several analogues substituted for nicotine in self-administration protocols with rats, they also decreased their lever-pressing to get nicotine, consistent with the hypothesis that partial agonists could be useful in smoking cessation.

*denotes the possible presence of additional, unidentified subunits, according to the recommendations of the IUPHAR nomenclature subcommittee on nAChR [Lukas, R.J. *et al.* (1999) *Pharmacol. Rev.* 51, 397–401]

Current advances in drug development

Several new ligands were described, either as lead compounds, or currently undergoing preclinical or clinical trials for Alzheimer's disease, Parkinson's disease, ADHD, schizophrenia and pain. This research has concentrated on the development of subtype-selective agonists, primarily for α4β2, α3β4* and α7* nAChR. Several compounds are being evaluated for use in Alzheimer's disease. These drugs have been targeted to several different nAChR subtypes. Jack Gordon (Astra Arcus, Worcester, MA, USA) described a potent α7-selective agonist, ARR17779, with low potency and efficacy in a dopamine-release assay, neuroprotective properties *in vitro* and beneficial effects in memory models *in vivo*. However, this compound is 20-fold less potent in chicken preparations than in the rat, and such species preferences could jeopardize its potency in humans. SIBIA have produced SIB1553A, described by Kenneth Lloyd (SIBIA), which is in Phase I clinical trials for Alzheimer's disease and displays selectivity for β4-containing nAChR.

Merouane Bencherif (Targacept) described several agonists targeted at α4β2 nAChR, namely RJR1734, RJR2403 (metanicotine) and RJR2557 (a metanicotine derivative). These compounds are being evaluated for cognitive enhancement and neuroprotection. Metanicotine is also antinociceptive in acute, persistent and chronic pain models and, according to Imad Damaj, is more selective than nicotine. Although not discussed at this meeting, Abbott Laboratories has reported that ABT594, a structural analogue of epibatidine, is a potent antinociceptive agent [Bannon, A.W. *et al.* (1998) *Science* 279, 77–80].

Pain management is clearly a major focus for nAChR therapy.

Because nAChR modulate the release of dopamine and stimulate locomotor behavior, Parkinson's disease is another therapeutic target for nicotinic drugs. David McClure (SIBIA) described SIB1508Y, a 5′-alkyne derivative of nicotine. This agonist appears to be selective for α4β2 nAChR and is in Phase II clinical trials for Parkinson's disease. When given to rats at the same time as they received a 6-hydroxydopamine lesion of the nigrostriatal tract, there was some sparing of the tyrosine-hydroxylase positive neurones. In an MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) monkey model of Parkinson's disease, SIB1508Y reputedly potentiated the effects of low and subthreshold doses of L-DOPA (3,4-dihydroxy-L-phenylalanine).

Future prospects

Through the evaluation of nAChR diversity, the development of agonists demonstrating improved subtype selectivity, and the demonstration of encouraging physiological and behavioural results, nAChR seems to be firmly established as an appropriate target for the treatment of a spectrum of conditions. Improved understanding of the specific phamacophore for each of the nAChR subtypes should lead to more selective agonists. Furthermore, greater knowledge of the subunit composition and distribution of nAChR subtypes expressed in the human brain, their functional properties and physiological significance will facilitate a more rational approach to ameliorating dysfunction. As most of the applications of nicotinic agents are likely to require long-term administration, questions of tolerance development and consequent changes in receptor numbers and function need to be addressed. So far, most research has been directed towards the development of agonists. Given the complex balance between receptor activation, desensitization, inactivation and regulation, evaluation of the effects of partial agonists and antagonists might be an alternative focus, while the allosteric modulation of nAChR is an attractive potential therapeutic approach. Steroids, peptides, local anaesthetics and antidepressants can modify receptor activity and further research of allosteric mechanisms is clearly warranted.

This conference provided a useful forum for dialogue between researchers in drug discovery programmes in industry and basic scientists in academia. While the former depends on breakthroughs in the fundamental understanding of nAChR structure and function that come, predominantly, from the endeavours of the academic community, the latter will benefit from the desperately needed new and selective research tools that are arising through the development of new drugs. This symbiosis will stimulate basic nAChR research and nicotinic therapeutics in tandem, and great advances can be anticipated for the next IBC conference on neuronal nAChR!

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Book review

Antifolate Drugs in Cancer Therapy

by Ann L. Jackman, Humana Press, 1999. $145.00 (456 pages, hardback), ISBN 0-89603-596-4

Antifolates are compounds that interfere with various stages of folate metabolism. There is an absolute requirement for folate coenzymes in cell division and tissue growth in mammals. Hence, antifolates are useful in regulating tissue growth and proliferation as exemplified by various neoplastic diseases. In addition, they can be used in the treatment of microbial infections, inflammatory disorders and autoimmune diseases. Four decades have elapsed since the introduction of methotrexate (MTX) into the clinic for the treatment of cancer, but its major therapeutically relevant metabolites were only discovered in 1973 [Baugh, C.M., Krumdieck, C.L. and Nair, M.G. (1973) *Biochem. Biophys. Res. Commun.* 52, 27–31; Nair, M.G. and Baugh, C.M. (1973) *Biochemistry* 12, 3923–3928]. This important and interesting discovery dramatically rekindled research efforts in this field [Cover legend (1992) *Cancer Res.* April 15]. The proceedings of the first two symposia pertaining to the role of polyglutamylation in antifolate cytotoxicity are still the most comprehensive reports of the early work and led to the development of the antifolates currently used in the clinic [Goldman, I.D., Chabner, B.A. and Bertino, J.R., eds (1983) in *Folyl and Antifolyl Polyglutamates*, Plenum Press; Goldman, I.D., ed. (1985) in *Proceedings of the Second Workshop on Folyl and Antifolyl Polyglutamates*, Preager Scientific].

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