EDITORIAL

Nicotinic Acetylcholine Receptors

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ABSTRACT

This themed issue of the *British Journal of Pharmacology* is being published in connection with a conference that focussed on nicotinic acetylcholine receptors (nAChRs) that was held on the Greek island of Crete from 7-11th May 2017. 'Nicotinic acetylcholine receptors 2017' was the fourth in a series of triennial international meetings that have provided a regular forum for scientists working on all aspects of nAChRs to meet and to discuss new developments. In addition to many of the regular participants, each meeting has also attracted a new group of scientists working in a fast-moving area of research. This themed issue comprises both review articles and original research papers on nAChRs.

LINKED ARTICLES

This article is part of a themed section on Nicotinic Receptors. To view the other articles in this section visit http://onlinelibrary/wiley.com/doi/

Abbreviations

LGIC, ligand-gated ion channel; nAChR, nicotinic acetylcholine receptor;

Nicotinic acetylcholine receptors (nAChRs) have been major players in the history of pharmacology, from Langley's initial concept of a 'receptive substance' in 1905 (see Changeux, 2012), to Paton and Zaimis's pharmacological distinction of muscle and ganglionic nAChRs (Paton & Zaimis, 1949), and Neher and Sakmann's functional analysis of nAChRs at the single channel level (which was recognised with the 1991 Nobel Prize in Physiology & Medicine and is reviewed in this themed issue by Bouzat & Sine, 2018). As the best characterised members of the family of ligand-gated ion channel (LGIC), nAChRs also became the protypical exemplar for the family of pentameric LGICs, including receptors for γ-aminobutyric acid ($GABA_A$Rs), glycine (GlyRs), 5-hydroxytryptamine (5-HT_3Rs) and glutamate (invertebrate GluCl receptors), as well as prokaryotic ancestors (Changeux, 2012).

The last 40 years or so have seen a shift of focus in nAChR research with the recognition that distinct 'neuronal nAChRs' occur in the CNS. The study of brain nAChRs was initially driven by the desire to understand nicotine dependence, responsible for tobacco smoking and its associated disease burden. Increasing awareness of the diversity of neuronal nAChRs, comprised of subunits arising from a distinct family of genes from those expressed in skeletal muscle (Alexander et al., 2017), has stimulated a broader interest in nAChRs, leading to drug discovery programmes for selective nicotinic therapeutics aimed at a wide range of neurological and mental health conditions. The subunit diversity and stoichiometry of nAChRs, and the associated pharmacology and clinical implications, are reviewed by Wang & Lindstrom (2018). Ten years ago, the burgeoning research into nAChRs inspired the first in a series of conferences, namely 'nAChRs 2008', hosted by the Wellcome Trust at Hinxton, Cambridge, UK. The success of this initial conference has been followed up every 3 years, with the latest conference, 'nAChRs 2017', being held in Crete in May 2017. The conference in Crete was a forum for presenting the latest developments in understanding the diversity, structure, function and clinical importance of nAChRs, with studies ranging from molecular to behavioural pharmacology.

Structural and functional diversity of nAChRs

A consequence of subunit diversity for pentameric receptors is the generation of multiple heteromeric receptor subtypes, where differences in a single subunit can have profound or subtle influences on receptor pharmacology and channel function. Wang & Lindstrom (2018) provide an authoritative account of the complex pharmacology arising from distinct stoichiometries of neuronal nAChRs. They compare the contributions of 'orthodox' nAChR agonist binding sites, at the interface between an α and β subunit, with 'unorthodox' sites that occur between two adjacent α subunits. The mechanistic influence of the fifth subunit, present between two orthodox agonist binding sites, is explored in the research paper by New et al. (2018).

In addition to the two classes of orthosteric site, nAChRs present numerous allosteric binding sites that allow either positive or negative modulation of agonist-evoked responses (Wang & Lindstrom, 2018). Positive allosteric modulators have excited considerable interest as therapeutic candidates; the clinical implications of the considerable pharmacological heterogeneity of nAChRs are briefly reviewed. Pharmacological complexity is further increased by considering the speed and duration of nAChR desensitisation. Papke and colleagues (2018) compare a novel positive allosteric modulator and a 'silent desensitiser' (that converts the α7 nAChR into a desensitised state without detectable receptor activation). Both elicit long-lasting, but distinct, non-conducting conformations of the nAChR.

The α3β2 and α3β4 nAChRs are the predominant subtypes found in the peripheral nervous system, and also occur in the brain and in non-neuronal cells. The exquisite pharmacological specificity of α-conotoxins, peptide toxins from marine cone snails, makes them attractive probes for discriminating nAChR subtypes and α-conotoxins selectively targeting α3β4 nAChR subtypes are discussed by Cuny et al. (2018). Computer modelling and molecular dynamic simulations reveal the molecular basis for the ability of certain conopeptides to discriminate between α3-containing nAChR subtypes, which could form the basis for custom-designed selective ligands.

Important considerations in the generation of multiple nAChR subtypes are the factors that govern subunit assembly and trafficking of nAChRs. A timely review of this topic is provided by Gotti and colleagues (Crespi et al., 2018), covering both endogenous protein chaperones, including RIC3, the Ly6 prototoxin family and the recently described NACHO, and exogenous pharmacological chaperones, notably nicotine.

Molecular structure and function

Drilling down to the inter-molecular details of agonist recognition, the molecular architecture of orthosteric ligand binding sites derived from the recently elucidated first crystal structures of the neuronal nAChR is reviewed by Giastas et al. (2018). These authors consider the structural basis of pharmacological differences between nAChR subtypes, focussing on human nAChR isoforms and drawing comparisons with the wider family of pentameric LGICs. They also discuss functionally important interactions between structural elements of the neuronal nAChRs that seem to be conserved across the LGIC superfamily and are important for coupling agonist binding to channel opening. Functional measurements at the molecular level are provided by electrophysiological recordings of individual nAChRs. In an erudite review of this methodology, Bouzat & Sine (2018) also consider the effects of subunit stoichiometry and allosteric modulation. Another approach to modulating receptor activity is through photosensitive ligands that can act as optical switches; Bregestovski and colleagues (2018) review the history, applications and limitations of photo-switches in LGIC research.

Clinical implications

Because of the pervasive influence of the neurotransmitter acetylcholine in central, peripheral and non-neuronal systems, it is not surprising that the cholinergic system is implicated in many disease states or their therapies. The generally modulatory nature of nAChRs residing outside of the neuromuscular junction and autonomic synapses make them attractive therapeutic targets for enhancing or decreasing cholinergic transmission or tone. In the CNS, depression has been linked to increases in cholinergic activity, and the high prevalence of tobacco smoking among persons suffering from depression has led to the suggestion that depressed patients may try to use nicotine to dampen cholinergic signals, through nAChR desensitisation. Consistent with this hypothesis, nAChR antagonists have some efficacy in animal models of depression. Picciotto and colleagues explored the role of α7 nAChRs in the hippocampus in anxiety- and depression-like behaviours in mice (Mineur et al., 2018). Pharmacological antagonism or local knockdown of α7 nAChRs (using short hairpin RNAs) produced some significant amelioration in certain mouse models but, interestingly, also revealed some striking gender differences.

Pain management is an area of great clinical need that has attracted much interest in the efficacy of nicotinic interventions. McIntosh and colleagues review the case for α9-containing nAChRs (α9*nAChRs) as therapeutic targets for treating neuropathic pain (Hone et al., 2018). The analgesic effects of certain α-conotoxins with high specificity for inhibiting α9*nAChRs, and the decreased pain sensitivity of α9 knockout mice, support a role for this nAChR subtype. However, the review is careful to point out inconsistencies in some research findings. The presence of α9*nAChRs in immune cells introduces immune cell-mediated inflammation as a potential mechanism to explain the efficacy of α9*nAChR blockade, and α9*nAChR-selective small molecule antagonists are being characterised.

A change in hippocampal α7 nAChRs was observed in chronically nicotine-treated mice subjected to wheel running, described in a research paper from Bailey and colleagues (Keyworth et al., 2018). The purpose of this study was to evaluate the effect of exercise during chronic exposure to nicotine (via implanted minipump) on the severity of subsequent precipitated withdrawal. Wheel running reduced withdrawal symptoms and increased α7 nAChRs in nicotine-treated (but not saline-treated) mice, while μ-opioid and D2 dopamine receptors were unaffected. These data implicate α7 nAChRs in the mechanism whereby exercise reduces subsequent nicotine withdrawal effects. It is interesting that exercise is also effective in countering depressive behaviours.

Another study into the effects of chronic nicotine on brain nAChRs, this time α4β2 nAChRs, was directed at determining the effect of gene dosage on consequent nAChR upregulation (Moretti et al., 2018). It is well established that chronic nicotine in vivo promotes the upregulation of α4β2 nAChRs in rodent brain, mimicking changes observed in the post mortem brain of tobacco smokers. Heterozygous mice lacking either one α4 allele or one β2 allele, or lacking one allele of each subunit, were compared with wild type mice for upregulation of α4β2 nAChRs following chronic nicotine administration. α4 subunit heterozygotes produced a disproportionate degree of upregulation compared with β2 heterozygotes and wild type mice (Moretti et al., 2018), suggesting that α4 polymorphisms that reduce α4 expression might produce enhanced responses to chronic nicotine, with implications for developing tobacco addiction.

Non-neuronal nAChRs

Nicotinic receptors are not limited to vertebrate nervous systems and the neuromuscular junction. 'Neuronal nAChRs' are also expressed by many non-neuronal cells including glia and immune cells, as already mentioned (Cuny et al., 2018; Hone et al., 2018). This non-neuronal locus challenges concepts of what acetylcholine and nAChRs do, as well as warranting a more inclusive nomenclature! Two research papers in this themed issue provide evidence that nAChRs expressed by cancer cells can drive proliferation. Mucchietto et al., (2018) report that nicotine (100 nM) promotes proliferation of lung adenocarcinoma cell lines via α7- and α9-containing nAChRs, and suggest these nAChRs mediate pathophysiological effects of tobacco smoke in non-small cell lung carcinoma. Consistent with this model, SLURP1 and SLURP2, endogenous peptide antagonists of α7 and non-α7 nAChRs respectively, are reported to decrease the rate of cell proliferation in a variety of epithelial cancer cell lines, in the study of Lyukmanova et al., (2018).

Insect nAChRs

Nicotinic receptors also have important roles in invertebrates. They are the major vehicle for excitatory transmission in insect nervous systems, making them important targets for the agrochemical industry, particularly in areas of crop protection. This has led to the development of novel classes of insecticides such as the neonicotinoids (see Cartereau et al., 2018). In this research paper, Steeve Thany and colleagues examine the effects of 3 neonicotinoids on mammalian α7 nAChRs. Their results show that these compounds are not potent agonists at rat α7 nAChRs, in contrast to their actions on nAChRs in insects (both pest and pollinating species), but novel modulatory actions were revealed. Molecular details of the interaction of neonicotinoids with Drosophila Dα1 subunit-containing nAChRs were examined by computer modelling, mutagenesis and oocyte electrophysiology, by Ihara et al. (2018).

Concluding remarks

This collection of reviews and research papers on nAChRs illustrates the breadth and calibre of current research into these receptors. The more we learn about their detailed molecular structures and mechanisms, and their physiological activities and contributions to disease models, so new questions and challenges are raised. The therapeutic potential of nAChRs remains to be fully exploited, but better understanding of the pharmacological manipulation of nAChRs will underpin the development of new nicotinic solutions for diverse clinical conditions. There is much to do and we can anticipate further exciting developments for future nAChR conferences.

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Conflict of interest
Authors of this editorial wish to acknowledge that they are co-authors of the articles by New et al. (IB) and Giastas et al. (ST) in this themed issue.

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