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Nicotinic acetylcholine receptors and the regulation of neuronal signalling

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Neuronal nicotinic acetylcholine (nACh) receptors in the brain are more commonly associated with modulatory events than mediation of synaptic transmission. nACh receptors have a high permeability for Ca^{2+}, and Ca^{2+} signals are pivotal in shaping nACh receptor-mediated neuromodulatory effects. In this review, we consider the mechanisms through which nACh receptors convert rapid ionic signals into sustained, wide-ranging phenomena. The complex Ca^{2+} responses that are generated after activation of nACh receptors can transmit information beyond the initial domain and facilitate the interface with many intracellular processes. These mechanisms underlie the diverse repertoire of neuronal activities of nicotine in the brain, from the enhancement of learning and memory, to addiction and neuroprotection.

The nicotinic acetylcholine (nACh) receptor is a ligand-gated channel that delineates a cation-selective pathway across the plasma membrane [1]. Comprising pentameric combinations of homologous, genetically distinct subunits, 'neuronal' nACh receptors are formed from a portfolio of α- and β-subunits (α2–α10 and β2–β4), the differential association of which confers distinct functional and structural properties to the resultant subtypes of nACh receptor [2,3].

nACh receptors mediate fast synaptic transmission in ganglionic neurons. However, there are only a few examples of this in the mammalian CNS [4,5] where nACh receptors exert a more modulatory influence [6]. In addition to rapid changes in membrane potential, activation of ligand-gated ion channels can also generate longer-lasting effects in the receptive neuron, which contribute to the elaboration of complex intracellular signals that mediate medium- to long-term events. In particular, the role played by intracellular Ca^{2+} signals in the survival of developing neurons, the modulation of their activity and, ultimately, their demise, places Ca^{2+}-permeable nACh receptors in a pivotal position for regulating such events [6]. In this review, we summarize current views on how neuronal nACh receptors govern Ca^{2+} signals and some of their downstream effectors.

nACh receptors and Ca^{2+} signals

Ca^{2+} permeability of nACh receptors

Traditionally, the Ca^{2+} permeability of neuronal nACh receptors has been studied by determining ionic reversal potential shifts, which allows estimation of the relative Ca^{2+}/Na^{+} permeability [7]. However, this approach does not always give a reliable quantitative estimate of Ca^{2+} permeability; a more direct, accurate estimation of fractional Ca^{2+} current, usually indicated as Pf, is provided by the correlation of whole-cell currents with fluorescence-based Ca^{2+} measurements [7]. Despite the apparent heterogeneity of available data, it is established that the subunit composition of nACh receptors influences their intrinsic Ca^{2+} permeability. Heteromeric neuronal nACh receptors comprising α- and β-subunits have a fractional Ca^{2+} current of 2–5% [7]. However, incorporation of the α5-subunit into α3-subunit-containing human nACh receptors, for example, significantly increases Ca^{2+} permeability [8]. Of all nACh receptors, homomeric α7 nACh receptors have the highest fractional Ca^{2+} current, which ranges from 6% to 12%, depending on the species [7,9]. Indeed, the fractional Ca^{2+} current through human α7 nACh receptors is the highest reported for homomeric ligand-gated receptors, and matches that of heteromeric NMDA receptors [10,11].

The generation of Ca^{2+} signals

The Ca^{2+} permeability of nACh receptors implies that a significant proportion of the nACh receptor-mediated increase in Ca^{2+} arises from direct permeation of the receptor channel. However, nACh receptor-mediated depolarization can activate voltage-operated Ca^{2+} channels (VOCCs), which augments the primary Ca^{2+} signals generated by nACh receptors [12,13]. These two mechanisms can be physiologically complementary: Ca^{2+} entry through inwardly rectifying nACh receptor channels will be greatest under either resting or hyperpolarized conditions, whereas Ca^{2+} influx through VOCCs only occurs at more depolarizing potentials (> -40 mV) [14]. This relationship might also apply to interactions between nACh receptors and NMDA receptors.

In addition to entry of extracellular Ca^{2+} through channels in the plasma membrane, the cytoplasmic concentration of Ca^{2+} reflects a complex interplay between buffering and mobilization capacities. In particular, Ca^{2+} release from intracellular stores can have a crucial role in defining Ca^{2+} responses [15]. In neuronal models,

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activation of Ca^{2+} stores following stimulation of nACh receptors contributes to long-lasting Ca^{2+} signals (Figure 1) [13,16–19]. Specific subtypes of nACh receptor seem to be associated with defined Ca^{2+} pathways: nACh receptors that contain α3- and/or β2-subunits in brain and ganglionic neuronal preparations are associated predominantly with Ca^{2+} signals that are mediated by depolarization and activation of VOCCs [13,17,18]. Although capable of activating VOCCs [20,21], α7 nACh receptors can also generate demonstrable Ca^{2+} transients independently of VOCCs. These transients reflect Ca^{2+} entry through the nACh receptor channel itself, which can then activate Ca^{2+}-induced Ca^{2+} release (CICR) from ryanodine-dependent stores (Figure 1) [13,17,22]. For example, in neurons of the substantia nigra pars compacta, depletion of internal Ca^{2+} stores inhibits the increase in cytoplasmic Ca^{2+} evoked by nicotine and the α7 nACh receptor-selective agonist choline [17]. Blockade of ryanodine receptors in neuroblastoma cells also significantly reduces the increase in cytoplasmic Ca^{2+} evoked by activation of β2- and α7-subunit-containing nACh receptors (Figure 1c) [13]. However, whereas the response of β2-subunit-containing nACh receptors depends entirely on activation of VOCCs, a proportion of the α7 nACh receptor-mediated increase in Ca^{2+} results from direct activation of ryanodine receptors without the intervention of VOCCs [13]. Functional coupling between α7 nACh receptors and ryanodine receptors has also been observed in hippocampal astrocytes (Figure 1d). In these cells, α7 nACh receptor-mediated Ca^{2+} signals arise primarily from CICR through ryanodine receptors [22]. In this elegant study, the authors took advantage of the ability of Sr^{2+}, but not Ba^{2+}, to substitute for Ca^{2+} in triggering CICR, to provide further evidence of the contribution of CICR to signalling initiated

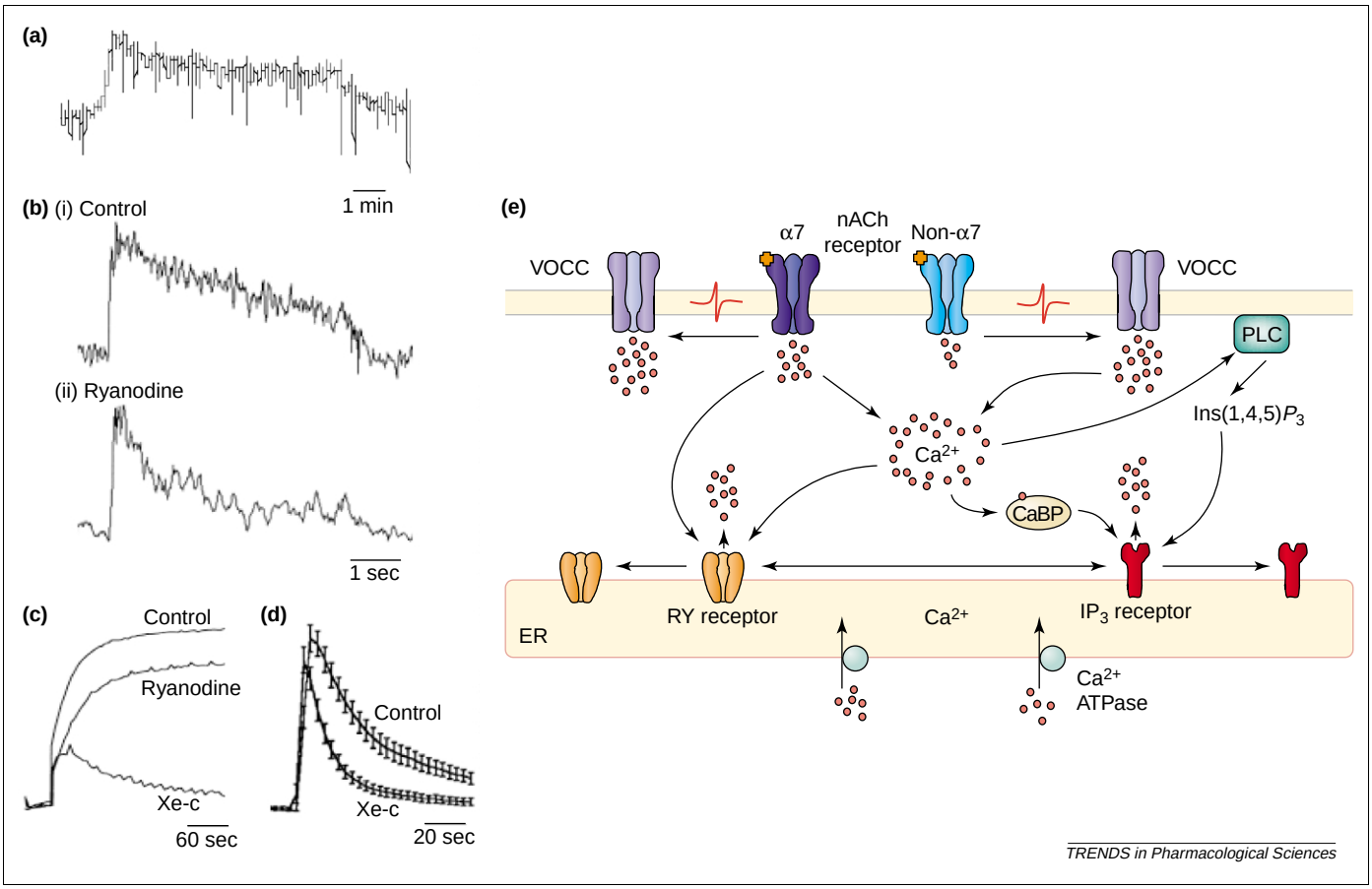

Figure 1. Nicotinic acetylcholine (nACh) receptor stimulation increases intracellular Ca^{2+} levels via many routes. The measurement of Ca^{2+} transients using Ca^{2+}-sensitive fluorescent dyes is depicted. **(a)** Nicotine (10 μM for 5 min) produces a sustained increase in cytoplasmic Ca^{2+} in hippocampal neurons loaded with fura-2. Reproduced, with permission, from [82]. **(b)** (i) nACh receptors mediate synaptically driven Ca^{2+} elevations in the on-spine region of a neuron from a chick ciliary ganglion loaded with Oregon green and stimulated (50 Hz) through the preganglionic nerve root. (ii) In the same preparation, 10 μM ryanodine, which blocks ryanodine-receptor-dependent release from Ca^{2+} stores, decreases the later phase of the nACh receptor-mediated, synaptically driven elevation of Ca^{2+}. Reproduced, with permission, from [18]. ©2001 Society for Neuroscience. **(c)** Ryanodine (30 μM) also reduces the nicotine-stimulated increase in cytoplasmic Ca^{2+} in human neuroblastoma cells loaded with fluo-3, as does the inositol (1,4,5)-trisphosphate [Ins(1,4,5)P_3] receptor blocker xestospongin-c (Xe-c,10 μM), which has more dramatic effects. Reproduced, with permission, from [13]. **(d)** nACh receptor-mediated increases in cytoplasmic Ca^{2+} in astrocytes loaded with fluo-3 after a short stimulation with acetylcholine (2 sec) are also reduced following incubation with Xe-c. In neuroblastoma cells and astrocytes, Ins(1,4,5)P_3 receptor-regulated Ca^{2+} release contributes more to the later phase of the nACh receptor-mediated response. Reproduced, with permission, from [22]. ©2001 National Academy of Sciences USA. **(e)** Generation of nACh receptor-dependent Ca^{2+} signals. Data indicate that nACh receptors augment the increase in intracellular Ca^{2+} that occurs by direct permeation of the nACh receptor channel by recruiting Ca^{2+} from several sources. These include voltage-operated Ca^{2+} channels (VOCCs), which might be activated as a consequence of membrane depolarization following nACh receptor activation. In turn, Ca^{2+}-induced Ca^{2+} release from internal stores is mediated by ryanodine (RY) receptors and Ins(1,4,5)P_3 (IP_3) receptors. Ins(1,4,5)P_3 might be generated by Ca^{2+}-dependent activation of phospholipase C (PLC). Alternatively, specific Ca^{2+}-sensor proteins (CaBPs) can activate Ins(1,4,5)P_3 receptors [26]. Different subtypes of nACh receptor might be coupled preferentially to specific sources of Ca^{2+}. For example, most nACh receptor subtypes that do not contain α7-subunits substantially amplify intracellular Ca^{2+} responses by activating VOCCs. However, α7 nACh receptors, which are highly permeable to Ca^{2+}, appear to be coupled functionally to direct activation of RY receptor-dependent stores. Thus, nACh receptor activation can generate sustained Ca^{2+} signals by the sequential activation of RY receptor- and Ins(1,4,5)P_3 -receptor-dependent Ca^{2+} stores.

by α7 nACh receptors. Although functional coupling implies a specific spatial relationship between neuronal nACh receptors and ryanodine receptors, this has not yet been demonstrated.

Activation of the inositol (1,4,5)-trisphosphate [Ins(1,4,5)P_3] second-messenger system and the subsequent release of Ca^{2+} from intracellular stores also contributes to intracellular Ca^{2+} signals (Figure 1). Although modulation of Ins(1,4,5)P_3 levels in response to activation of nACh receptors was demonstrated in muscle cells in culture more than a decade ago [23], the involvement of Ins(1,4,5)P_3-receptor-dependent Ca^{2+} stores in neuronal nACh receptor signalling has been reported only recently, with the demonstration that nACh receptor-evoked Ca^{2+} responses are reduced in the presence of selective Ins(1,4,5)P_3 receptor antagonists (Figure 1c,d) [13,22,24]. The functional interaction between Ins(1,4,5)P_3 and ryanodine receptor-dependent Ca^{2+} signals is considered to be a key signalling mechanism [15]. Reports that nACh receptor-evoked release of Ca^{2+} from Ins(1,4,5)P_3 receptors is secondary to that from ryanodine receptors are consistent with their sequential activation [13,22]. Although it is unclear how nACh receptor stimulation activates Ins(1,4,5)P_3 receptors, plausible mediators are a Ca^{2+}-dependent phospholipase C [25] and/or Ca^{2+}-sensor proteins [26] activated following nACh receptor stimulation.

The ability to activate different sources of Ca^{2+} confers a further spatial and temporal dimension to the Ca^{2+} signals evoked by nACh receptor activation (Figure 1). By converting acute nACh receptor stimulation into sustained cellular events, Ca^{2+} signals are the crucial link between nACh receptors and the downstream processes that impinge on many neuronal functions. In turn, nACh receptor activity is regulated by levels of intracellular Ca^{2+} [27,28], indicative of a complex reciprocal relationship.

nACh receptors and the modulation of neuronal function

Although neuronal nACh receptors are major mediators of fast synaptic transmission in the PNS [29,30], both electrophysiological and transmitter-release studies support the view that a significant proportion of nACh receptors in the CNS are presynaptic [6,31]. However, somatodendritic nACh receptors (not necessarily postsynaptic) occur [4,5,28,32–34], and nACh receptors at both loci shape the physiological consequences of nACh receptor activation.

Regulation of neurotransmitter release

Presynaptic nACh receptors facilitate the Ca^{2+}-dependent release of many neurotransmitters, which is consistent with activation of exocytotic mechanisms [31]. In addition to promoting exocytosis by opening VOCCs through membrane depolarization, nACh receptors can also initiate exocytosis directly by virtue of their intrinsic Ca^{2+} permeability. There is evidence for the operation of both mechanisms. For example, in striatal dopamine synaptosomes bearing β2-subunit-containing nACh receptors [35], nACh receptor-evoked release of dopamine is mediated by VOCCs [36,37]. By contrast, activation of $\alpha3\beta4^*$ nACh receptors on hippocampal synaptosomes evokes the release of [^{3}H]noradrenaline without the involvement of VOCCs [37]. Because the Ca^{2+} permeabilities of these native receptors are unknown [7], it is uncertain if this difference reflects their intrinsic permeability or their association with VOCCs. Presynaptic α7 nACh receptors at excitatory synapses can increase the probability of glutamate release in the presence of tetrodotoxin and VOCC blockers [38]. Further insights into this relationship have come from a recent study that shows that Ca^{2+} entry through α7 nACh receptors on hippocampal mossy fibre terminals initiates CICR from presynaptic stores, which elicits bursts of miniature excitatory postsynaptic currents [39]. As a result, the stimulation of presynaptic nACh receptors might boost postsynaptic activity to levels that affect the properties of neuronal networks [40].

In addition to provoking Ca^{2+}-dependent exocytosis, presynaptic nACh receptors also modulate transmitter release through Ca^{2+}-mediated cellular mechanisms. For example, protein kinase C (PKC) has a proposed role in the modulation of striatal dopamine release by nACh receptors [41], and nACh receptor and PKC-mediated stimulation of extracellular signal-regulated mitogen-activated protein kinase (ERK/MAPK) [42] and annexin phosphorylation [43] are reported to contribute to the regulation of exocytosis in adrenomedullary cells.

Regulation of gene expression

The conversion of electrical to biochemical signals through local increases in cytoplasmic Ca^{2+} and activation of Ca^{2+}-sensitive proteins allows the rapid transduction of synaptic information to the nucleus. As an example, somatodendritic nACh receptors can also influence transmitter release by modulation of gene expression. The best-characterized example is the regulation of tyrosine hydroxylase (TH) expression. As the rate-limiting step in catecholamine biosynthesis, TH is a major control point in neurotransmitter release from catecholamine-containing neurons, and is subject to diverse regulatory mechanisms [44]. Long-term treatment with nicotine elevates the concentration of TH mRNA and, consequently, TH activity, both *in vivo* and in chromaffin cells [44]. This effect is Ca^{2+} dependent and mediated by protein kinase A [45]. The nicotine-evoked induction of expression of the gene encoding TH requires a prolonged increase in Ca^{2+} concentrations, and activation of store-dependent Ca^{2+} channels [45] and ERK/MAPK [46]. Given that α7 nACh receptors are implicated in activating expression of the gene that encodes TH [24], these results assume an extra significance in view of the proposed association between this nACh receptor subtype and intracellular Ca^{2+} stores (Figure 1).

In addition to influencing neurotransmitter release, a role for nACh receptors in the regulation of cell signalling and gene expression has been apparent for some time [47]. Initial studies with nicotine focused on the immediate early genes c-*Fos* and c-*Jun* [47,48], and the advent of gene-array technology facilitates identification of novel downstream targets of nACh receptor activation. For example, in neuroblastoma cells, exposure to nicotine

influences the expression of a diverse set of genes, including transcription and protein-processing factors, and proteins associated with RNA binding and the plasma membrane [49]. Identification of novel gene targets introduces the challenge of elucidating the signalling pathways through which nACh receptors regulate their expression. During the past decade many mediators that provide vital pieces in the cell-signalling jigsaw have been reported (Figure 2). Although still fragmentary, some examples illustrate the current perspective on nACh receptor-mediated signalling and its targets, with potential implications for physiological and/or pathological mechanisms.

Plasticity events and memory mechanisms

Agonists of nACh receptors can improve, and nACh receptor antagonists impair, performance in cognitive tasks [50], which implicates nACh receptors in cognitive function. Elucidating the cellular mechanisms that underlie the contribution of nACh receptors is a daunting task, but recent studies highlight potentially key mechanisms. The cAMP response element-binding protein (CREB) and ERK/MAPK signalling cascades have attracted particular attention because their activities are central to long-term plasticity in the nervous system [51]. This might have physiological relevance to many functions, including addiction, learning and memory [52].

nACh receptors mediate the Ca^{2+}-dependent activation of ERK/MAPK and CREB in several neuronal models [53–55]. Notably, the hippocampus has received particular attention as a key area for memory processing, and nACh receptor- and store-mediated Ca^{2+} influx promotes activation of Ca^{2+}–calmodulin-dependent protein kinase and ERK/MAPK, and the sustained phosphorylation of CREB [55,56]. In addition, presynaptic α7 nACh receptors enhance the probability of long-term potentiation in hippocampal preparations [57] and might also contribute to the mechanisms that underlie the effects of nicotine on cognition.

Activation of ERK/MAPK is required for the formation of contextual and spatial memories in mammals [51]. Thus, factors that interfere with the activation of this pathway by α7 nACh receptors might contribute to cognitive decline. It is notable that the β-amyloid peptide ($A\beta_{1-42}$), which accumulates in Alzheimer's disease, binds specifically and potently to α7 nACh receptors [58]. Moreover, $A\beta_{1-42}$ functionally blocks the current responses evoked by activation of α7 nACh receptors in either hippocampal neurons [59] or following heterologous expression in *Xenopus* oocytes [60,61]. Although one study reports that picomolar concentrations of $A\beta_{1-42}$ activate whole-cell currents in oocytes that express nACh receptors [62], others have failed to observe this [59,61]. Recently, activation by $A\beta_{1-42}$ of whole-cell and single-channel recordings from rat basal forebrain neurons has been reported, but, paradoxically, nACh receptors that do not contain α7-subunits are implicated [63]. Picomolar concentrations of $A\beta_{1-42}$ also elicit α-bungarotoxin-sensitive

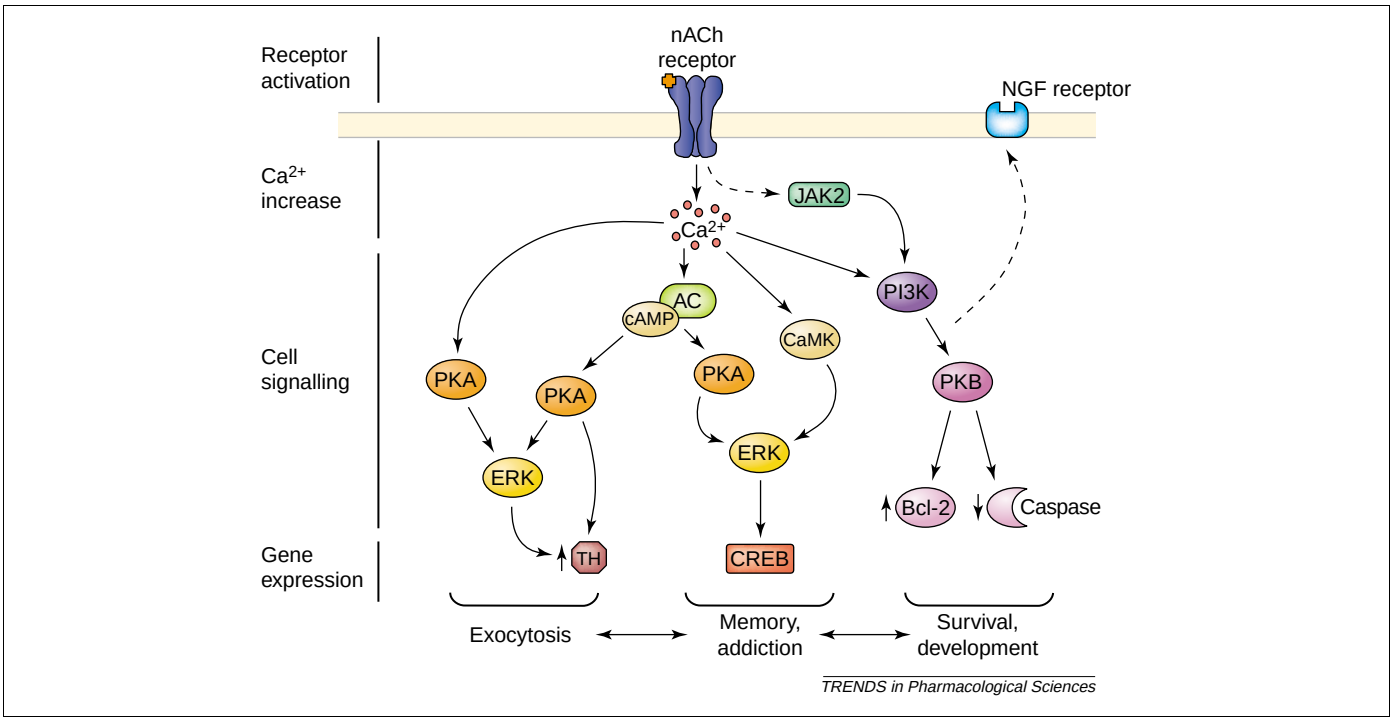

Figure 2. Key signalling molecules in Ca^{2+}-dependent, nicotinic acetylcholine (nACh) receptor-mediated neuronal processes (for clarity, some intermediate steps are not shown). The capacity of nACh receptors to regulate diverse neuronal functions depends on the activation of specific signalling cascades. Thus, the increase in intracellular Ca^{2+} that arises from nACh receptor activation can activate adenylyl cyclase (AC), protein kinase A (PKA), PKC, Ca^{2+}–calmodulin-dependent protein kinase (CaMK) and phosphatidylinositol 3-kinase (PI3K). In turn, these phosphorylate downstream targets, such as extracellular signal-regulated mitogen-activated protein kinase (ERK), which leads to the activation of transcription factors such as the cAMP response element-binding protein (CREB) and increases in expression of genes that encode, for example, tyrosine hydroxylase (TH) and nerve growth factor (NGF) receptors. The lipid signalling cascade that is initiated by PI3K, through phosphorylation of PKB (Akt), is credited with modulating the relative activities of neuroprotective and apoptotic factors, such as Bcl-2 and caspases, respectively [87,88]. In addition, Ca^{2+}-dependent phosphatases are also likely to be recruited [85], but little is known about nACh receptor-regulated phosphatase activity. Thus, nACh receptors can exert a wide range of influences through Ca^{2+} signals, from changes in synaptic plasticity, which is pertinent to many situations including cognition, memory and addiction, to the life-and-death events involved in development and neuroprotection. Abbreviation: JAK, janus-activated kinase.

increases in intracellular Ca^{2+} in synaptosomes [64], but it is uncertain if this is a direct consequence of nACh receptor activation. An important caveat is that the physical state of the $A\beta_{1-42}$ used in most of these *in vitro* studies (soluble and nonaggregated) might not be relevant pathologically.

The long-term effects of $A\beta_{1-42}$ are more representative of the situation in Alzheimer's disease; chronic exposure of rat hippocampal slices to $A\beta_{1-42}$ results in upregulation of α7 nACh receptor protein *in vitro* [65]. *In vivo*, in transgenic mice expressing a human mutant form of amyloid precursor protein that is anticipated to result in overexpression of $A\beta_{1-42}$, the increase in the level of α7 nACh receptors correlates with downregulation of ERK/MAPK and phosphorylated CREB [65]. In addition, animals with elevated levels of α7 nACh receptors show learning and memory deficits, purported to reflect defective ERK/MAPK and CREB signaling as a result of $A\beta_{1\text{-}42}$ activation and consequent desensitization of α7 nACh receptors [65,66]. However, ERK/MAPK lies at the convergence of many signalling pathways, and so a particular association with α7 nACh receptors in relation to the behavioural changes, while attractive, is still unproven. Indeed, this highlights a major challenge: to close the knowledge gap that exists between discrete molecular and cellular mechanisms and behaviour [67]. Nevertheless, the recent report that $A\beta_{1\text{-}42}$ promotes hyperphosphorylation of the microtubule-associated protein Tau, the other pathological hallmark of Alzheimer's disease, via α7 nACh receptors and the ERK/MAPK signalling cascade [68] supports a central role for α7 nACh receptor signalling in the progression of cognitive diseases.

Reward and dependence

Drug dependence is considered to involve plastic changes in circuits that are associated with 'rewarding' behaviours. Nicotine dependence, which is mediated by interaction with nACh receptors, is likely to involve the modification of signalling cascades that modulate synaptic plasticity and gene expression, as proposed for other drugs of abuse [52,69]. Like other addictive substances and rewarding behaviours, nicotine increases the release of dopamine from mesolimbic projections to the nucleus accumbens (NAc) [70]. Although somatodendritic nACh receptors on dopamine-containing neurons of the ventral tegmental area (VTA) can excite these neurons directly, which results in transient responses that are terminated by desensitization of nACh receptors [71], the stimulation and subsequent desensitization of VTA GABA-containing neurons also contributes to this excitatory effect through removal of the inhibitory influence of GABA [72]. In addition, burst firing and sustained release of dopamine from mesolimbic neurons in response to nicotine depends on concomitant stimulation of NMDA receptors in the VTA [73]. Glutamate-containing afferents to the VTA synapse with dopamine-containing neurons, and presynaptic α7 nACh receptors on glutamatergic terminals are thought to facilitate glutamate release [74]. Indeed, in rat brain slices that contain both VTA and NAc, it has been demonstrated [75] that activation of presynaptic α7 nACh receptors induces long-term potentiation of the excitatory input to the VTA if nicotine application is paired with postsynaptic stimulation. Significantly, this modification of synaptic function is reminiscent of the mechanisms that are postulated to contribute to learning and memory in other areas of the brain.

Although there are few reports on the contributions of signalling pathways in models of nicotine addiction, several studies have focused on the modulation of CREB following chronic treatment with nicotine, often with dissimilar results. Pandey *et al.* [76] showed that, in rats, nicotine withdrawal (but not chronic treatment with nicotine itself) significantly reduced the concentrations of CREB and phosphorylated CREB in rat cortex and amygdala; phosphorylated CREB also decreased in the NAc in mice following chronic consumption of nicotine in their drinking water [77]. Changes in phosphorylated CREB in the NAc are consistent with previous reports that decreased CREB activity in this region contributes to drug reinforcement [78]. The view that common substrates mediate the addictive properties of different drugs in the mesocorticolimbic system [79] highlights the possibility that such responses might not be mediated directly by nACh receptor-initiated signalling cascades. Instead, they might occur in downstream neurons: for example, in the GABA-containing neurons of the NAc that receive dopamine inputs from the VTA. Thus, in striving for a molecular and cellular account of behavioural phenomena, it is necessary to distinguish primary and secondary signalling events that are initiated by activation of nACh receptors.

Neuroprotection and the regulation of cell death

Nicotine and other nACh receptor agonists are neuroprotective in several models of neuronal death, both *in vivo* and *in vitro* [80]. Although the intracellular steps that mediate the neuroprotective effects of nACh receptor ligands are not solved fully, nACh receptor-mediated neuroprotection against excitotoxicity is Ca^{2+} dependent [81–83] and does not involve blockade of glutamate receptor function [82–84]. Thus, activation of downstream signalling pathways appears necessary for the prevention of neuronal death, but there is no consensus on the preeminence of any particular cascade. In hippocampal slices, nicotine-mediated protection against acute NMDA damage is mediated by the activation of phosphatidylinositol 3-kinase (PI3K) and ERK/MAPK [83]. These signalling molecules could increase the expression of Ca^{2+} buffering proteins such as calbindin-D28K, which have been implicated in the nACh receptor-dependent amelioration of excitotoxic insults [84]. In cortical cultures, nicotine-induced, Ca^{2+}-dependent activation of the phosphatase calcineurin is proposed to mediate the protection afforded by nicotine against glutamate excitotoxicity [85]. Stimulation of nACh receptors can also lead to the increased expression of neurotrophic factors and nerve growth factor receptors *in vitro* and *in vivo* [86]. This capacity to enhance trophic signalling might reflect the role ascribed to nACh receptors in neurodevelopmental processes [6]. Studies in which nicotine prevents Aβ-induced neurotoxicity in primary cortical cultures indicate an additional mechanism of nACh receptor-mediated

neuroprotection, through Janus kinase 2, PI3K, phosphorylation of Akt (PKB) and upregulation of the anti-apoptotic marker Bcl-2 [87,88]. This is consistent with our studies and those of others, which demonstrate that stimulation of nACh receptors prevents neuronal loss through a decrease in caspase activity and inhibition of apoptosis [89,90].

The many signalling pathways implicated to date might reflect the dynamic, interactive nature of these processes, in addition to differences between type of preparation, insult and nACh receptor stimulation. Significantly, some of the protective anti-apoptotic mechanisms that are activated in neuronal models of cell death can have deleterious effects in peripheral, mitotic cells, where inhibitors of apoptosis can act as tumour promoters. Indeed, stimulation of nACh receptors has anti-apoptotic effects in several transformed cell types [91,92], and, *in vivo*, peripheral effects of nicotine stimulate angiogenesis and promote tumour growth through a reduction in apoptosis [93].

Concluding remarks

nACh receptors can generate specific, complex Ca^{2+} signals (Figure 1) that influence several signalling molecules and neuronal processes (Figure 2). The combination of neuronal type, developmental stage, nACh receptor subtype, Ca^{2+} signals and signalling pathway translates the initial nACh receptor stimulation into a neuronal response and, ultimately, a physiological outcome. The structural basis of signal specificity is likely to depend on the complement of scaffolding proteins and their associated activities, which are anticipated to interact with nACh receptor subunits through their large cytoplasmic domains. Recent studies have identified interactions with known scaffolding and adaptor proteins, including 14–3–3η [94] and members of the PSD-95 family [95,96], and provide the first insights into the structural framework through which nACh receptor-mediated signalling is organized. Thus, the concept that nACh receptors are ligand-gated cation channels that only generate fast electrical signals is being supplanted by a more complex scenario in which nACh receptors are fundamental players in the elaboration of spatially and temporally complex neuronal signals.

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