Chapter 1
Overview

Akinori Akaike and Yasuhiko Izumi

Abstract The nicotinic acetylcholine receptor (nAChR) is a typical ion channel type receptor. nAChR agonists such as nicotine evoke rapid excitatory responses in order of milliseconds. In addition to acute responses, sustained stimulation of nAChRs induces delayed cellular responses leading to neuroprotection via intracellular signal pathways probably triggered by Ca^{2+} influx. The most predominant subtypes of nAChRs expressed in the central nervous system (CNS) are α4 (known as α4β2) and α7 nAChRs. Long-term exposure to nicotine or acetylcholinesterase (AChE) inhibitors exerts protection against neurotoxicity induced by glutamate, β-amyloid, and other toxic insults. Nicotinic neuroprotection is mediated by α7 nAChR which shows high Ca^{2+} permeability, though contribution of α4 nAChR to nicotinic neuroprotection has also been suggested. Agonist stimulation of these receptors leads to activation of the phosphoinositide 3-kinase (PI3K)-Akt signaling pathway, downstream of neurotrophin receptors. AChE inhibitors including donepezil which is used for treatment of Alzheimer's disease, also activate PI3K-Akt pathway via nAChRs. Neuroprotective effects induced by long-term nAChR stimulation indicate that CNS nAChRs play important roles in promotion of neuronal survival under pathophysiological conditions such as brain ischemia and neurodegenerative diseases. Elucidation of neuroprotective mechanisms of nAChRs may enable development of novel therapies for neurodegenerative diseases.

Keywords Acetylcholine · Acetylcholinesterase · Neuroprotection · Nicotine · Nicotinic

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1.1 Introduction

Acetylcholine (ACh) is a small molecule with a simple chemical structure comprising an ester of choline and acetic acid. This molecule plays a crucial role in maintaining homeostasis and brain functions by acting as a neurotransmitter in the peripheral nervous system including motor nerves and the autonomic and the central nervous system (CNS). ACh is synthetized by choline acetyltransferase with choline and acetyl coenzyme A as substrates (Fig. 1.1). ACh released from nerve endings upon nerve excitation is rapidly degraded by acetylcholinesterase (AChE) into choline and acetic acid. ACh released in the synaptic cleft acts as an agonist to its specific receptors to evoke various cellular responses. ACh receptors are divided into two major classes, nicotinic ACh receptors (nAChRs) and muscarinic ACh receptors (mAChRs). The names of these receptors are derived from their specific agonists; nicotine contained in tobacco leaves and muscarine isolated from poisonous mushrooms, *Amanita muscaria*. nAChRs are ligand-gated ion channels, which evoke rapid depolarization responses to elicit neuronal excitation or skeletal muscle contraction. On the other hand, mAChRs are representative G-protein-coupled receptors classified as M_1–M_5 (Caulfield and Birdsall 1998). M_1, M_3, and M_5 receptors interact with Gq-type G proteins and primarily cause excitatory responses, whereas M_2 and M_4 receptors interact with Gi/Go type G proteins and cause suppressive responses such as hyperpolarization. Responses mediated by mAChRs are relatively slow whereas opening of ligand-gated channels of nAChRs induces rapid cellular responses in the order of milliseconds.

nAChRs are highly expressed in skeletal muscle and the nervous system. Recently, expression of nAChRs in immune cells and glial cells has also attracted attention for potential therapeutic targeting in inflammation and neurodegenerative diseases (de Jonge and Ulloa 2007; Fujii et al. 2017; Jurado-Coronel et al. 2016).

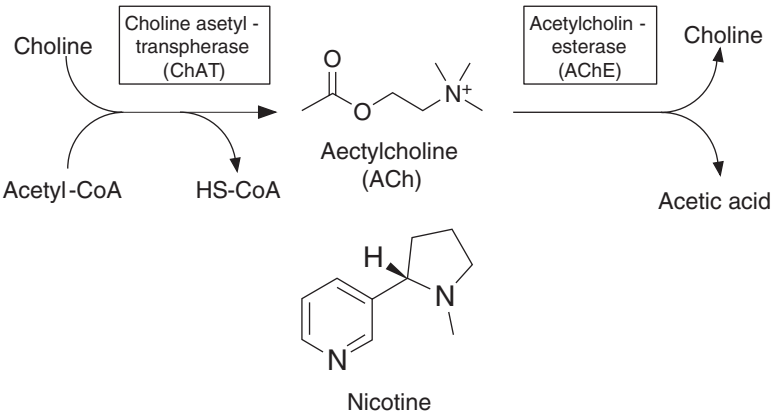

Fig. 1.1 Synthesis and metabolism of acetylcholine (ACh). Choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) are involved in synthesis and metabolism of ACh. ACh is synthesized from Acetyl coenzyme A (Acetyl-CoA) and Choline, releasing Coenzyme A (HS-CoA)

nAChRs are grouped into muscle-type (N_m), peripheral neuronal-type (N_n), and central neuronal-type (CNS) based on their distribution, subunit composition, and selective antagonists, as per the classification in Goodman & Gilman's "The Pharmacological Basis of Therapeutics" (12th Edition, 2011). In their classification, CNS AChRs are further divided into two subtypes: $(\alpha4)_2(\beta2)_3$ (α-bungarotoxin-insensitive) and $(\alpha7)_5$ (α-bungarotoxin-sensitive). N_n AChRs are widely expressed in autonomic ganglia and the adrenal medulla. CNS AChRs are expressed in neurons and glia of various brain areas. One of the typical antagonists of N_m AChRs is *d*-tubocurarine, a toxic alkaloid derived from an arrow poison and clinically used as a non-depolarizing blocking agent of the neuromuscular junction. Hexamethonium and mecamylamine are selective antagonists of N_n and CNS AChRs.

In all types of nAChRs, agonists such as ACh itself or nicotine-induced ion channel opening and evoke influx of Na^+ and Ca^{2+}. This triggers cell depolarization and turns on various functional switches (Albuquerque et al. 2009). Nicotinic cholinergic responses correlated with fast neurotransmission are easily detected in the endplate at the neuromuscular junction and ganglion cells of the sympathetic nerves. By contrast, it is relatively difficult to detect postsynaptic nicotinic responses of neurons in the CNS because most neuronal nAChRs quickly desensitized when exposed to nicotinic agonists (Albuquerque et al. 2009; Alkondon et al. 1998; Frazier et al. 1998). Development of drug-delivery devices that allow fast drug delivery and removal has made it possible to detect fast responses mediated by functional CNS nAChRs. While peripheral nAChRs are involved in rapid responses such as skeletal muscle contraction, nAChRs expressed in the CNS tend to be involved in relatively slow functional changes. For example, in the cerebral cortex, persistent nAChR stimulation triggers signals to the phosphoinositide 3-kinase (PI3K) cascade, which contributes to neuroprotection (Kihara et al. 2001; Dajas-Bailador and Wonnacott 2004). In the hippocampal neurons, nAChRs induce long-term potentiation of synaptic transmission (Kenney and Gould 2008). nAChRs regulate dopamine release in the striatum (Exley and Cragg 2008). Moreover, nAChRs are one of the important factors regulating memory and addiction (Molas et al. 2017; Nees 2015). Thus, in addition to rapid responses such as membrane depolarization induced by inward currents via ion channels, nAChR can generate longer-lasting effects in the CNS neurons, where rapid cation influx may trigger activation of complex intracellular signaling pathways.

1.2 Structural and Pharmacological Characterization of Nicotinic Acetylcholine Receptors

nAChRs are classified as members of the cysteine-loop (Cys-loop) family of ligand-gated ion channels (Sine and Eagle 2006; Tsetlin et al. 2011). The Cys-loop ligand-gated channels, also known as Cys-loop receptors, play prominent roles in generating excitatory and inhibitory postsynaptic potentials in the nervous system. nAChRs,

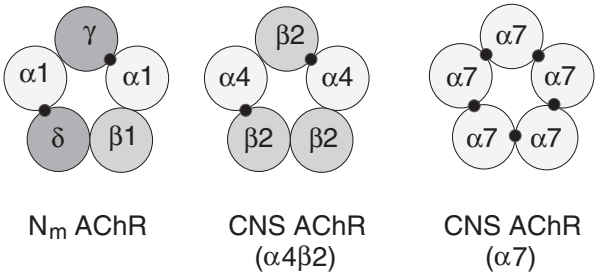

Fig. 1.2 Examples of subunit assembly and location of agonist-binding sites. Large circles indicate subunits of nicotinic acetylcholine receptor (nAChR). Small filled circles indicate binding sites of acetylcholine. Muscle-type AChR (N_m AChR), central nervous system AChR (CNS AChR)

γ-aminobutyric acid type A ($GABA_A$) receptors, glycine receptors, and 5-hydroxytryptamine type-3 ($5\text{-}HT_3$) receptors are classified as Cys-loop receptors. These receptors are composed of five subunits, forming a pentameric conformation around a central water-filled pore. The Cys-loop receptors have structurally common features with a characteristic loop formed by a disulfide bond between two cysteine residues. In nAChRs, the two cysteine residues separate 13 highly conserved amino acids located in the extracellular N-terminal domain of the α-subunit. The four hydrophobic transmembrane domains are estimated to form α-helices that make up the ion channel pore. The channel pore is lined with residues from the second transmembrane domain (TM2) from each of the five subunits of the receptors. The extracellular domain is largely composed of the N-terminus with binding sites for agonists.

The International Union of Basic and Clinical Pharmacology Committee on Receptor Nomenclature and Drug Classification (NC-IUPHAR, URL: http://www.guidetopharmacology.org/nciuphar.jsp) recommends a nomenclature and classification scheme for nAChRs based on subunit composition of known, naturally occurring and/or heterologously-expressed nAChR subtypes. A total of 17 subunits (α1–10, β1–4, γ, δ, and ε) have been identified in nAChRs. All subunits except α8, which is present in avian species, have been identified in mammals. ACh-binding sites are found at interfaces of the α subunit and the δ or γ subunit in N_m AChRs, and at interfaces of the α subunit and β subunit or two adjacent α subunits in N_n and CNS AChRs (Fig. 1.2). All α subunits possess two tandem cysteine residues near the ACh-binding site. By contrast, β, γ, δ, and ε subunits lack these cysteine residues. N_m AChRs of adult animals possess the stoichiometry $(\alpha1)_2\beta1\delta\varepsilon$ while N_m AChRs expressed in embryonic muscles and denervated adult muscles possess the stoichiometry $(\alpha1)_2\beta1\gamma\delta$ (Lukas et al. 1999). Other types of nAChRs are predominantly expressed in neurons (Table 1.1). They are assembled as combinations of α2–α6 and β2–β4 subunits or α7, α8, and α9 subunits forming functional homo-oligomers. N_m AChRs and some subtypes of CNS AChRs (α7, α8, α9, and α10) are sensitive to α-bungarotoxin, a well-known neurotoxic protein derived from the venom of kraits.

Table 1.1 Characteristics of nAChR

Subtype	Primary subunit composition	Ca^{2+} permeability	Major location	α-Bungarotoxin sensitivity
α1	$(\alpha1)_2\beta1\gamma\delta$, $(\alpha1)_2\beta1\delta\varepsilon$	Low	Neuromuscular junction	Sensitive
α2	α2β2, α2β4	Low	CNS	Insensitive
α3	α3β2, α3β4	Low	Autonomic ganglion, CNS	Insensitive
α4	$(\alpha4)_3(\beta2)_2$, $(\alpha4)_2(\beta2)_3$	Low	CNS	Insensitive
α5	α3β2α5, α3β4α5, $(\alpha4)_2(\beta2)_2\alpha5$	High	Autonomic ganglion, CNS	Insensitive
α6	α6β2β3, α6α4β2β3	High	CNS	Insensitive
α7	$(\alpha7)_5$	High	CNS, Non-neuronal cells	Sensitive
α8 (avian only)	$(\alpha8)_5$	High	CNS	Sensitive
α9	$(\alpha9)_5$, α9α10	High	Mechanosensory hair cells	Sensitive
α10	α9α10	High	Mechanosensory hair cells	Sensitive

For α2, α3, α4, and β2 and β4 subunits, pairwise combinations of α and β (e.g., α3β4 and α4β2) are sufficient to form a functional receptor in vitro, but more complex isoforms may exist in vivo. Among those subunit combinations, the α3β4 subunit combination is dominant in nAChRs of autonomic ganglia neurons. The α5 and β3 subunits participate in formation of functional hetero-oligomeric receptors when they are expressed as a third subunit with another α and β pair such as α4α5αβ2, α4αβ2β3, and α5α6β2. The α6 subunit can form a functional receptor when co-expressed with β4 in vitro. The α7 subunit forms functional homo-oligomers. This subunit can also combine with a β subunit to form a hetero-oligomeric assembly such as α7β2. The α8 and α9 subunits show similar properties to the α7 subunit. For functional expression of the α10 subunit, co-assembly with α9 is necessary.

Subtypes of nAChRs can be classified based on the predominant α-subunits (α1–α10) because the α subunit plays a key role in agonist binding to trigger ion channel opening, and subtype-selective antagonists like α-bungarotoxin distinguish receptors based on the α subunit combination (see Table 1.1). As per this receptor classification, N_m AChRs can be defined as α1 nAChRs, because the α1 subunit is highly expressed only in skeletal muscle and other α subunits are not detected in this tissue. N_n and CNS AChRs can be broadly classified into two subgroups, α2–α6 nAChRs, formed from the combination of α- and β-subunits, and α7–α9 nAChRs, forming homo-oligomers. The former subgroup, α2–α6 nAChRs, is insensitive to α-bungarotoxin whereas the latter subgroup, α7–α9 nAChRs, is sensitive to the toxin. Ion channels of homo-oligomeric receptors α7–α9 show high Ca^{2+} permeability. The α5 and α6 hetero-oligomeric receptors also show high Ca^{2+} permeability.

Table 1.2 Distribution of nAChR in CNS

α2	α3	α4	α5	α6	α7
Cortex		Cortex	Cortex		Cortex
Hippocampus	Hippocampus	Hippocampus	Hippocampus		Hippocampus
		Striatum	Striatum	Striatum	
Amygdala		Amygdala			Amygdala
		Thalamus			
Hypothalamus		Hypothalamus			Hypothalamus
	Substantia nigra	Substantia nigra	Substantia nigra	Substantia nigra	Substantia nigra
	Cerebellum	Cerebellum			Cerebellum
	Spinal cord	Spinal cord			Spinal cord

Among those neuronal receptors, α3 nAChR is highly expressed in autonomic ganglia though this subtype is also expressed in CNS. The most predominant subtypes of nAChRs expressed in CNS are α4, known as α4β2 and α7 nAChRs (Dani 2015). Expression of both subunits is detected across wide areas of the CNS (Table 1.2). In the cerebral cortex, α2 and α5 subunits are also detected. Accumulating evidence also suggests anti-inflammatory and neuroprotective roles of α7 nAChR expressed in immune cells and glial cells (Egea et al. 2015; Morioka et al. 2015).

1.3 Neuroprotection Mediated by Nicotinic Acetylcholine Receptors

It is widely recognized that glutamate acts as an excitatory neurotransmitter but also exerts excitatory neurotoxicity in pathological conditions such as ischemia (Meldrum and Garthwaite 1990; Duggan and Choi 1994; Brassai et al. 2015). In addition to cerebral ischemia, glutamate neurotoxicity is also considered as one of the risk factors for neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease. Involvement of the cholinergic system in glutamate neurotoxicity was first reported in Mattson's study (1989), showing that glutamate neurotoxicity in the hippocampus was enhanced by mAChR stimulation. Olney et al. (1991) showed evidence suggesting that *N*-methyl-D-aspartate (NMDA) receptor blockade by MK801 induces disinhibition of the central cholinergic system and causes excessive stimulation of mAChRs. They hypothesized that MK801 occasionally induces neurotoxicity instead of neuroprotection due to such an indirect mAChR stimulation. Thus, it is likely that mAChRs facilitate neuronal death in pathological states where glutamate neurotoxicity causes neurodegeneration.

On the other hand, accumulating evidence has suggested that nAChRs play a protective role in glutamate neurotoxicity. Approximately two decades ago, Akaike et al. (1994) and Kaneko et al. (1997) reported that glutamate neurotoxicity in the

cerebral cortex was suppressed by nicotine and other nAChR agonists. Because NMDA receptors are acknowledged as a predominant route of glutamate cytotoxicity in the cerebral cortex, nicotine was suggested to prevent glutamate neurotoxicity by exerting a protective action against NMDA receptor-mediated intracellular responses to induce neuronal death. The neuroprotective effect of nicotine was antagonized by hexamethonium and mecamylamine, which are N_n and CNS nAChR antagonists, respectively, indicating that nicotine induces neuroprotection by its selective action on nAChRs. To our knowledge, our study (Akaike et al. 1994) was the first evidence for the neuroprotective role of nAChRs in the CNS. In this study, nicotine markedly reversed glutamate cytotoxicity, whereas muscarine exacerbated it. Carbachol, which acts on both nicotinic and muscarinic receptors, reduced glutamate cytotoxicity although its effect was less potent than that of nicotine. These observations indicate that nAChRs and mAChRs exert opposing effects on glutamate cytotoxicity. Moreover, findings of nAChR-mediated neuroprotection suggested a role of nicotinic cholinergic system in promoting neuronal survival under pathological conditions such as brain ischemia. A characteristic feature of the neuroprotective action of nicotine was that long-term exposure of more than an hour was necessary to ameliorate glutamate neurotoxicity. Following our findings in the cerebral cortex, neuroprotective effects mediated by nAChRs have been detected in various areas of the brain, including the hippocampus (Dajas-Bailador et al. 2000; Liu and Zhao 2004), the striatum (Ohnishi et al. 2009), dopaminergic neurons in the substantia nigra (Takeuchi et al. 2009), and the spinal cord (Nakamizo et al. 2005; Toborek et al. 2007). Nicotinic neuroprotection detected in those studies is estimated to be mediated by nAChR expressed in neurons though contribution of microglia activation by α7 nAChR in nicotinic neuroprotection is also suggested (Morioka et al. 2015).

It is unlikely that nicotine-induced protection against glutamate neurotoxicity is due to its direct action on NMDA receptors though there are some reports indicating that nicotine partially inhibits NMDA receptors. Aizenman et al. (1991) have demonstrated that nicotinic agonists partially inhibit whole cell NMDA-induced responses in cultured cortical neurons. Akaike et al. (1991) also reported modulatory action of cholinergic drugs on NMDA responses in the nucleus basalis of Meynert neurons. These studies suggest that nicotinic agonists have properties to directly interact with NMDA receptors and modulate their function. In this case, concomitant application of nicotine and glutamate or short-term nicotine exposure should affect glutamate neurotoxicity by direct modification of NMDA receptors. However, as described above, long-term exposure for more than an hour is necessary to detect nicotinic neuroprotection (Akaike et al. 1994; Kaneko et al. 1997). Moreover, nicotine-induced protection against glutamate cytotoxicity was antagonized by CNS nAChR antagonists. Therefore, persistent stimulation of nAChRs, but not direct inhibition of NMDA receptors is estimated to be the major route of nicotine-induced neuroprotection though direct interaction of nicotine with NMDA receptors may potentiate nicotine-induced neuroprotection.

In the forebrain including the cerebral cortex, α7 nAChRs, homo-oligomers of α7 subunits and α4β2 nAChRs, hetero-oligomers of α4 and β2 subunits are the major subtypes among CNS nAChRs (Albuquerque et al. 2009; Zoli et al. 2015). It has been reported that nicotine-induced protection against glutamate neurotoxicity was antagonized by selective α7 nAChR antagonists α-bungarotoxin and methyllycaconitine, as well as by the selective α4β2 nAChR antagonist dihydro-β-erythroidine (Kaneko et al. 1997). The α7 nAChR has attracted more attention because its mechanisms are thought to be involved in Alzheimer's disease and β-amyloid (Aβ), a well-known risk factor of Alzheimer's disease, is bound to α7 nAChRs under several conditions including in post-mortem Alzheimer's disease brains (Wang et al. 2000; Parri et al. 2011). A selective α7 nAChR agonist, 3-(2,4)-dimethoxybenzylidene anabaseine (DMXB), exhibits potent neuroprotective action on glutamate neurotoxicity in vitro and brain ischemia in vivo (Shimohama et al. 1998). Aβ-induced neurotoxicity was suppressed by nicotine and DMXB (Kihara et al. 1997). Protective effects of nicotine and DMXB against Aβ-induced toxicity were antagonized by α-bungarotoxin, indicating that stimulation of α7 nAChRs is essential in suppressing Aβ-induced neurotoxicity. It is widely accepted that the β sheet conformation of Aβ is necessary in eliciting its neurotoxicity (Fändrich et al. 2011). Nicotine might influence the β sheet conformation of Aβ to attenuate its toxicity or to modulate survival signals. However, it has been reported that neither nicotine nor DMXB influences the β sheet conformation (Kihara et al. 1999). Thus, signal transduction downstream of α7 nAChRs is likely to be involved in the protective effect of nicotine against Aβ neurotoxicity.

1.4 Intracellular Signal Transduction Triggered by Nicotinic Acetylcholine Receptors

On exposure to agonists, nAChR exists in an active, open state, and elicits rapid depolarization in order of milliseconds. Thus, nAChR is classified as an excitatory receptor that evokes rapid excitation in neuronal, muscular, and secreting cells. Progressive decline of agonist-evoked current indicates closure of the channel. Upon further exposure to agonists, nAChRs exist in desensitized, non-functional states. Besides such short-term response, it is also recognized that nAChRs mediate long-term modification of cell functions via specific signaling pathways (Dajas-Bailador and Wonnacott 2004). nAChRs, especially α7 nAChRs, generate specific and complex Ca^{2+} signals that include adenylyl cyclase, protein kinase A, protein kinase C, Ca^{2+}-calmodulin-dependent kinase, and phosphatidylinositol 3-kinase (PI3K) (Fig. 1.3). These phosphorylated downstream targets activate cellular signaling related to exocytosis and extracellular signal-regulated mitogen-activated protein kinase (ERK)-linked neuronal functions. Kihara et al. (2001) showed that α7 nAChR stimulation promoted PI3K-Akt signal transduction and inhibited Aβ neurotoxicity.

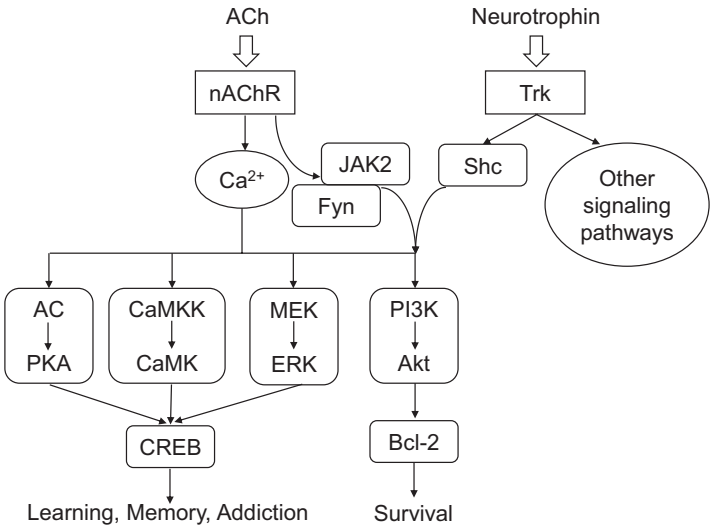

Fig. 1.3 Nicotinic acetylcholine receptor (nAChR)-mediated signaling pathway in the brain. Adenylate cyclase (AC), acetylcholine (ACh), nAChR, AKT8 virus oncogene cellular homolog (Akt), B-cell lymphoma 2 (Bcl-2), calcium/calmodulin-dependent protein kinase (CaMK), calcium/calmodulin-dependent protein kinase kinase (CaMKK), cAMP-responsive element binding protein (CREB), extracellular signal-regulated kinase (ERK), Fgr/Yes-related novel protein (Fyn), Janus-activated kinase (JAK), MAPK/ERK kinase (MEK), nicotinic acetylcholine receptor (nAChR), phosphoinositide 3-kinase (PI3K), protein kinase A (PKA), SH2-containing collagen-related proteins (Shc), tropomyosin receptor kinase (Trk)

PI3K phosphorylates Akt (or known as protein kinase B), a serine/threonine kinase. Activation of PI3-Akt cascade stimulates B-cell lymphoma 2 (Bcl-2) family members, which act as anti-apoptotic factors. It has been shown that Fyn, a member of the non-receptor type Src tyrosine kinase family, is associated with α7 nAChRs, though it is not clear whether other Src family members are involved in the cascade downstream of nAChRs. A relationship between nAChRs and Fyn was also implicated in a study, showing that catecholamine release induced by nicotine was dependent on the presence of Fyn and extracellular Ca^{2+} (Allen et al. 1996). In the study by Kihara et al. (2001), an inhibitor of Src tyrosine kinase reduced Akt phosphorylation. In addition, PI3K and Fyn were physically associated with α7 nAChRs. These findings suggest that nAChR stimulation causes Akt phosphorylation via signal transduction through Fyn to PI3K. Ca^{2+} influx through the α7 nAChR ion channels might contribute to this process. It has been proposed that PI3K-Akt activation leads to up-regulation of Bcl-2 to promote neuronal survival (Matsuzaki et al. 1999; Kihara et al. 2001).

The intracellular signal pathway downstream of CNS nAChRs is known as a major pathway of neuroprotective action of neurotrophins including nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) (Dajas-Bailador and Wonnacott 2004; Lim et al. 2008). NGF and BDNF are known to affect survival and

differentiation of central and peripheral neurons. The PI3K/Akt signaling cascades play a key role in neuronal survival due to neurotrophins (Chan et al. 2014). It has been reported that NGF and BDNF prevent glutamate neurotoxicity in a time-dependent manner, exhibiting significant neuroprotection in a period >1 h (Shimohama et al. 1993a, b; Kume et al. 1997, 2000). Each neurotrophin interacts with specific tropomyosin receptor kinase (Trk) receptors. Trk receptors show selectivity to members of the neurotrophin family. TrkA, TrkB, and TrkC serve as preferential receptors for NGF, BDNF, and neurotrophin-3, respectively (Kalb 2005). In contrast to these high-affinity receptors, the low-affinity neurotrophin receptor, p75, interacts with all neurotrophin members. BDNF promotes survival of neurons via TrkB in several brain regions including the cerebral cortex. Moreover, nAChRs appear to transduce survival signals similar to signals downstream of the Trk receptors of neurotrophins (Dajas-Bailador and Wonnacott 2004). Thus, nicotine and neurotrophins show similar properties in terms of time-course and signal pathways of neuroprotection.

1.5 Acetylcholinesterase Inhibitors Used for Treatment of Alzheimer' Disease

The finding that glutamate neurotoxicity is suppressed by continuous stimulation of nAChRs suggests a possible function of the nicotinic cholinergic system as a factor promoting neuron survival in the CNS. AChE inhibitors including donepezil, which easily permeates the blood–brain barrier, are used for Alzheimer's disease. Takada et al. (2003) reported that in cultured cortical neurons, AChE inhibitors including donepezil, galantamine, and tacrine inhibited glutamate neurotoxicity, though concomitant addition of AChE inhibitors and glutamate did not exhibit neuroprotection. Neuroprotective effects of AChE inhibitors were antagonized by N_n and CNS AChR antagonists including mecamylamine and methyllycaconitine, but not by a mAChR antagonist, scopolamine. Thus, AChE inhibitors appeared to possess neuroprotective effects similar to properties of nicotinic neuroprotection. AChE inhibitors such as donepezil remarkably suppress apoptosis of neurons induced by long-term administration of low concentrations of glutamate. Investigation of the involvement of PI3K on the protective action of AChE inhibitors revealed that the neuroprotective action of donepezil and galantamine is associated with Fyn, Janus Activating Kinase 2 (JAK2), and PI3K (Takada-Takatori et al. 2006; Akaike et al. 2010). In addition, these central AChE inhibitors promoted phosphorylation of Akt and increased the expression level of Bcl-2 protein. These results indicate that the PI3K-Akt signaling pathway is important for protection mechanisms of AChE inhibitors.

nAChRs are also recognized as major functional molecules mediating pharmacological action of tobacco smoking. Nicotine is a major ingredient of tobacco and stimulates all subtypes of nAChRs, though nicotine induces more rapid

desensitization of nAChRs than ACh (Albuquerque et al. 2009). Several clinical studies have shown a negative correlation between prevalence of sporadic Parkinson's disease and smoking history in relation to nAChR and neurodegenerative diseases, although no clear conclusion can be reached as to the relationship between Alzheimer's disease and smoking (Godwin-Austen et al. 1982; Tanner et al. 2002; Ulrich et al. 1997). Moreover, galantamine, possessing allosteric potentiating action on α7 nAChR, is used as a treatment for Alzheimer's disease (Albuquerque et al. 2001; Santos et al. 2002). Interestingly, long-term tobacco smoking or nicotine application induces up-regulation of nAChRs and, in most cases, facilitates their functions (Brody et al. 2013; Govind et al. 2009). This phenomenon is quite unique because, in most neuronal receptors including mAChRs, long-term receptor stimulation by specific agonists usually induces down-regulation of receptors and reduction of receptor functions. Moreover, AChE inhibitors including donepezil induce significant up-regulation of nAChRs (Kume et al. 2005; Takada-Takatori et al. 2010). Activation of the PI3-Akt pathway is necessary for nAChR up-regulation following long-term donepezil exposure. Receptor up-regulation following long-term exposure to nicotine and AChE inhibitors may be linked to diverse properties of nAChRs, from enhancement of learning and memory to addiction and neuroprotection, although precise mechanisms of up-regulation are not fully understood.

1.6 Conclusion

Nicotine induces fast nAChR currents of the order of milliseconds, while sustained nicotine exposure induces delayed intracellular responses. Neuroprotection is one of the dominant delayed responses mediated by CNS nAChRs. Mechanisms of neuroprotective effects exerted by persistent nAChR stimulation cannot be described only by simple excitatory reactions following depolarization induced by ion channel openings, but rather by activation of the intracellular PI3K-Akt signaling pathway leading to up-regulation of the anti-apoptotic protein Bcl-2. α7 nAChR, which shows high Ca^{2+} permeability, plays a crucial role in nicotinic neuroprotection. The metabolic change with Ca^{2+} as the second messenger may play an important role in triggering signals downstream of nAChRs. Therefore, it can be proposed that nAChRs are apparently implicated in two types of cellular functions; one for fast depolarization and the other for slow intracellular responses leading to neuroprotection (Fig. 1.4). Nicotine and other nAChR agonists evoke both acute and delayed responses; the former involves receptor desensitization and the latter involves receptor up-regulation. On the other hand, AChE inhibitors directly or indirectly stimulate nAChRs without evoking apparent acute responses (Akaike et al. 2010; Takada-Takatori et al. 2010). Neuroprotection and nAChR up-regulation by long-term exposure to AChE inhibitors, used in treatment of Alzheimer's disease, suggest that CNS nAChRs are an important component of defense mechanisms of neurons

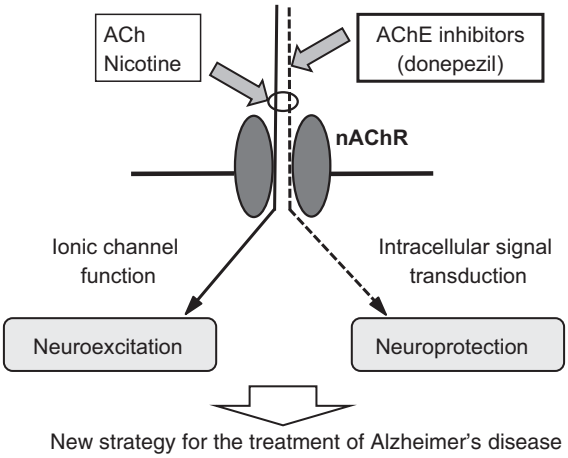

Fig. 1.4 Schematic representation of presumed roles of the nicotinic acetylcholine receptor (nAChR) in the central nervous system (CNS). Acetylcholine (ACh) and nicotine act on CNS nAChR to exert both neuroexcitation via ionic channel function and neuroprotection via intracellular signal transduction. Acetylcholinesterase (AChE) inhibitors such as donepezil exert neuroprotection without exhibiting neuroexcitation

against risk factors of neurodegeneration in pathophysiological conditions. Manipulation of neuroprotective properties of nAChRs may be a novel therapeutic approach for treatment of neurodegenerative diseases including Alzheimer's disease.

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