Chapter 27
α7 Nicotinic ACh Receptors as a Ligand-Gated Source of Ca^{2+} Ions: The Search for a Ca^{2+} Optimum

Victor V. Uteshev

Abstract The spatiotemporal distribution of cytosolic Ca^{2+} ions is a key determinant of neuronal behavior and survival. Distinct sources of Ca^{2+} ions including ligand- and voltage-gated Ca^{2+} channels contribute to intracellular Ca^{2+} homeostasis. Many normal physiological and therapeutic neuronal functions are Ca^{2+}-dependent, however an excess of cytosolic Ca^{2+} or a lack of the appropriate balance between Ca^{2+} entry and clearance may destroy cellular integrity and cause cellular death. Therefore, the existence of optimal spatiotemporal patterns of cytosolic Ca^{2+} elevations and thus, optimal activation of ligand- and voltage-gated Ca^{2+} ion channels are postulated to benefit neuronal function and survival. Alpha7 nicotinic acetylcholine receptors (nAChRs) are highly permeable to Ca^{2+} ions and play an important role in modulation of neurotransmitter release, gene expression and neuroprotection in a variety of neuronal and non-neuronal cells. In this review, the focus is placed on α7 nAChR-mediated currents and Ca^{2+} influx and how this source of Ca^{2+} entry compares to NMDA receptors in supporting cytosolic Ca^{2+} homeostasis, neuronal function and survival.

Keywords α7 nAChR • NMDA • Ca^{2+} • Permeability • Ion channel • Receptor • ACh • Choline • Nicotinic • Positive allosteric modulator • PNU-120596 • Neuroprotection • Cognitive • Cognition • Dementia • Neurotoxicity • Synaptic • Extrasynaptic • Alzheimer • Schizophrenia • Trauma • Auditory • Gating

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Ligand- and Voltage-Gated Sources of Ca^{2+} Ions

Background

Changes in cytosolic Ca^{2+} levels act as a messenger relaying information from the cellular membrane to the cellular cytoplasm and the nucleus. In neurons and other excitable cells, this message encodes the amplitude and duration of activation of voltage- and/or ligand-gated ion channels. The cellular response then includes a sequence of intracellular biochemical reactions that alter the expression and function of genes and proteins. In healthy neurons, the expression of different Ca^{2+} sources and the spatiotemporal patterns of Ca^{2+} entry are well-balanced and an adequate match between Ca^{2+} demand and supply is usually observed. However, when Ca^{2+} sources become dysfunctional due to age, disease, or trauma, persistent imbalance in Ca^{2+} entry and clearance destroys cellular integrity, leading to cellular damage, dysfunction, and excessive proliferation or death depending on the type of cells and the strength of the insult. Neuronal damage or loss may result in severe chronic neurodegenerative conditions including sensorimotor deficits and dementia. Therefore, a tight but subtle control of cytosolic Ca^{2+} levels is required for neuronal health, development and function. Understanding the pharmacology and mechanisms of cytosolic Ca^{2+} messaging is essential for developing successful preventative strategies and treatments for neurodegenerative conditions associated with aging, dementia and brain trauma.

Inadequate vs. Optimal Ca^{2+} Entries and Neuronal Fate

An important common motif in the livelihood of central neurons is the existence of an optimum in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) and the spatiotemporal patterns of cytosolic Ca^{2+} elevations. This optimum promotes neuronal survival and delivers functional benefits to neurons. The farther $[Ca^{2+}]_i$ is from its optimum, the greater is the likelihood of neuronal damage and death. Accordingly, excessive elevations in $[Ca^{2+}]_i$ mediated by excessive activation of ligand- and/or voltage-gated Ca^{2+} ion channels have been associated with a loss of neuronal function and neuronal death (see, for instance, [1–11]). Moreover, in a number of *in vivo* and *in vitro* experimental models of normal aging and Alzheimer's disease (AD), elevated levels of cytosolic Ca^{2+} have been linked to age- and disease-related dysregulations in the function of voltage-gated Ca^{2+} ion channels (VGCCs) and N-Methyl-D-Aspartate (NMDA) receptor-mediated ion channels [2, 3, 6, 7, 10–17]. Conversely, moderate elevations in $[Ca^{2+}]_i$, for example, via a K^+-induced depolarization or weak persistent activation of highly Ca^{2+}-permeable $\alpha 7$ nicotinic acetylcholine receptors (nAChRs) have been shown to protect neurons from death in a variety of toxicity models [18–28]. In addition, some biologically active compounds (e.g., estrogen,

insulin-related growth factor 1 and positive allosteric modulators of α7 nAChRs) potentiate Ca^{2+} permeable voltage- or ligand-gated ion channels and increase Ca^{2+} influx [29–37] which can be neuroprotective and cognitively beneficial.

Originally, the concept of excitotoxicity linked neuronal injury to excessive elevations in $[Ca^{2+}]_i$ which resulted from activation of a variety of Ca^{2+} sources including ligand- and voltage-gated Ca^{2+} ion channels [38]. As such, the "Ca^{2+} set-point" hypothesis was introduced, proposing four stages of neuronal responsiveness to elevation in $[Ca^{2+}]_i$ elicited by K^+-dependent depolarization or electrical stimulation [1, 22, 39]: (1) a lack of neuroprotection in the near absence of cytosolic Ca^{2+} regardless of neurotrophic support (stage 1); (2) neuronal survival in the presence of normal cytosolic Ca^{2+} (~100 nM) with neurotrophic support (stage 2); (3) neuronal survival in the presence of moderate elevation in cytosolic Ca^{2+} (~200 nM) regardless of neurotrophic support (stage 3) and (4) an excess (>1 μM) of Ca^{2+} and neuronal death (stage 4). Although the Ca^{2+} set-point hypothesis supported the concept of Ca^{2+} optimum for neuronal survival and function, it did not explain the role of specific pathways of Ca^{2+} entry leaving a key question unanswered: can an elevation in $[Ca^{2+}]_i$ be optimal regardless of the pathway of Ca^{2+} entry?

Role of NMDARs

Further studies revealed that elevations in $[Ca^{2+}]_i$ are derivatives of a more elementary chain of events consisting of Ca^{2+} entry and intracellular Ca^{2+} processing. According to this concept, neuronal fate (i.e., survival or death) is predominantly determined by the source of Ca^{2+} entry rather than $[Ca^{2+}]_i$ [40]: i.e., Ca^{2+} ions entering the cell via NMDARs are much more likely to cause damage to the cell than similar amounts of Ca^{2+} ions entering the cell via VGCCs. In fact, VGCC-mediated elevations in $[Ca^{2+}]_i$ are more likely to be neuroprotective than neurotoxic (see above and [1, 20, 22, 24, 39, 41]). However, moderate activation of NMDARs during preconditioning in low concentrations of glutamate (<50 μM) as well as activation of nAChRs by nicotine have also been found to promote neuronal survival (see below and [41–44]). In general, a proper investigation of neuroprotective and neurotoxic effects of individual Ca^{2+} sources requires selective pharmacological tools because multiple Ca^{2+} sources often act in conjunction resulting in a cumulative elevation in $[Ca^{2+}]_i$ and emergent response properties [45–48].

The NMDAR-dependent pathways of cytosolic Ca^{2+} regulation are complex as both excessive activation and blockade of NMDARs promote neuronal death [5, 49–51], while moderate activation of NMDARs is absolutely required for normal neuronal development and function. As a result, a key challenge in development of NMDAR-based therapies is introduced by a possibility that the same agent (e.g., NMDAR antagonist) or process (e.g., NMDAR activation) can be both neuroprotective and neurotoxic depending on the neuronal status and the phase, intensity and

duration of ongoing neuronal damage. Therefore, the therapeutic index (i.e., the ratio of the lethal dose to the therapeutic dose) of many NMDAR agents would be expected to be variable, case-dependent and ≤1 on average.

A pool of functional NMDARs can be subdivided into synaptic and extrasynaptic based on their location relative to the synaptic cleft. Recent studies have started to explore an intriguing possibility that activity of synaptic and extrasynaptic NMDARs defines neuronal fate [50, 51]: activation of synaptic NMDARs leads to neuroprotection, while activation of extrasynaptic NMDARs is neurotoxic. Therefore, the overall intensity of NMDAR activation may not be as defining for the fate of neurons as the fraction of synaptic vs. extrasynaptic NMDAR activation. According to this hypothesis, Ca^{2+} ions entering neurons through extrasynaptic NMDARs are the most harmful. The basis for differences between the effects of synaptic and extrasynaptic NMDARs is not well-understood, but may include at least three factors, as discussed by [50]: (1) differences in the intracellular signaling pathways; (2) differences in the NMDAR subunit composition; and (3) differences in the activation profiles (e.g., synaptic NMDARs are typically activated by high transient concentrations of synaptic glutamate (~1 mM); while extrasynaptic NMDARs are activated by persistent, but relatively low concentrations (≤1 μM) of ambient glutamate). However, the division of NMDARs into synaptic and extrasynaptic may be rather provisional because NMDARs can move laterally between synaptic and extrasynaptic sites [52]. This behavior is not unique to NMDARs and has also been observed in α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) [53] and α3-/α7-containing nAChRs [54].

Moreover, direct measurements of extracellular glutamate levels [55] as well as experimental and computer modeling of glutamatergic synaptic transmission and spillover [56–58] suggest that even after relocation to extrasynaptic sites (i.e., up to several micrometers away from presynaptic release site), NMDARs do not become independent of synaptic stimulation as they can still be activated by synchronous glutamate spillovers originating from multiple active glutamatergic synapses [59, 60]. The effectiveness of glutamate spillover in activation of extrasynaptic NMDARs and cross-talk between adjacent synapses directly results from morphological and release properties of central punctate glutamatergic synapses [56, 61] and kinetic properties of NMDARs: i.e., high potency (EC_{50} ~ 3 μM, [62]) and incomplete desensitization [63, 64]. Consistent with these views, the otherwise low levels of extracellular glutamate in hippocampal slices (e.g., ~25 nM; [65]) can be substantially enhanced in the vicinity of active glutamatergic synapses [55] or during the reversal of neuronal/glial glutamate transporters that may take place under ischemia and other pathological conditions [66, 67]. However, what happens to intracellular pathways linked to an individual receptor as it switches teams (i.e., from synaptic to extrasynaptic) remains unknown (see more discussions on this topic in [50, 68, 69]).

This apparent ambiguity in the role of NMDARs in neuronal death and survival should not derail the ongoing search for a therapeutic optimum in the level of NMDAR activation and Ca^{2+} entry while the fact that, to date, clinical trials have

been mostly unsuccessful in identifying effective NMDAR-based therapies against ischemia and other neurodegenerative conditions invites discoveries of new approaches and nontrivial solutions like never before. One of these promising emergent approaches termed "pathologically activated therapeutics" [70] makes use of low-potency open-channel NMDAR blockers, such as memantine [71]. These compounds may have neuroprotective properties as their inhibitory effects do not preclude the physiologically beneficial low-intensity activation of NMDARs, but substantially reduce the excessive activation of NMDARs which is neurotoxic. However, memantine has been also shown to inhibit α7 nAChRs with a similar or even greater potency (IC_{50} ~ 0.3–5 μM) than NMDARs (IC_{50} ~ 1–10 μM) [72–74]. In some cases, inhibition of α7 nAChRs by memantine may be counterproductive because moderate activation of α7 nAChRs is usually neuroprotective and cognitively beneficial (see below). Despite numerous reports of positive effects of memantine on patients with AD, non-AD dementias and other neurodegenerative disorders [75–81], the effectiveness, consistency and safety of memantine-based therapies have been questioned on multiple occasions [72, 82–85] and neurotoxic effects of therapeutic doses of memantine (~20 mg/kg) have been reported, for example, due to a drug interaction between memantine and common acetylcholine esterase inhibitors, such as donepezil [82, 86]. Accordingly, targeting intracellular sites downstream of NMDAR activation may present an alternative and possibly, more promising therapeutic approach [87].

α7 nAChRs

Background

Neuronal nicotinic AChRs are cation-selective and Ca^{2+} permeable ion channel complexes. Twelve genes encoding for neuronal nAChR subunits have been identified to date [88]. Four of these genes encode for α7, α8, α9, and α10 subunits that may form functional homomeric nAChRs when expressed alone. The family of functional heteromeric nAChRs is more diverse: these functional receptors are required to have two principal α subunits (i.e., α2, α3, α4 or α6) and two or three complementary β subunits (i.e., β2 or β4). In addition, one structural subunit (i.e., α5 or β3) may also be present [89]. Among nAChRs, the α7 nAChR exhibits the highest permeability ratio of Ca^{2+} over Na^{+} ions (P_{Ca}/P_{Na}) [90–97]. The high Ca^{2+} permeability of α7 nAChRs suggests important roles for this receptor in modulation of neurotransmitter release, gene expression, neuroprotection and neurotoxicity [98–101]. The existing evidence indicates that α7 nAChRs maintain a high degree of functional homology, including Ca^{2+} permeability, across species and preparations [102, 103]. Therefore, the properties of α7 nAChRs expressed in heterologous systems are expected to be comparable to native α7 nAChRs expressed in various

brain regions. However, although α7 nAChRs can form functional homomeric nAChRs, there is a growing pool of evidence for the existence of functional heteromeric α7-containing nAChRs resulting from co-expression of α7 and non-α7 subunits (e.g., α5, β2 and β3 subunits). These native α7-containing heteromeric receptor ion channel complexes exhibit pharmacological, kinetic and desensitization properties somewhat different from those of homomeric α7 nAChRs expressed in heterologous systems [104–112].

The early studies of Ca^{2+} permeability of α7 nAChRs used primarily heterologous systems expressing homomeric α7 nAChRs and reported the permeability ratios for Ca^{2+} over Na^{+} ions substantially greater than those for NMDARs: P_{Ca}/P_{Na} (α7R) ~ 15–20 vs. P_{Ca}/P_{Na} (NMDAR) ~ 8–10 [93–95, 113]. However, more recent studies used hippocampal cultured neurons and acutely dissociated hippocampal and hypothalamic neurons to report more modest values: P_{Ca}/P_{Na}(α7R) ~ 6 vs. P_{Ca}/P_{Na} (NMDAR) ~ 8–10 [90, 97]. Moreover, in these experiments the Ca^{2+} permeability of NMDARs was found to be significantly greater than that of α7 nAChRs [97]. The observed discrepancies between the early and more recent studies may have resulted from differences in agonist application techniques, data analysis and estimates of ionic activities and liquid junction potentials. Alternatively, it is possible that native, possibly heteromeric, α7-containing nAChRs exhibit a lower Ca^{2+} permeability than homomeric α7 nAChRs. However, a direct comparison of Ca^{2+} permeabilities of native and heterologous α7 nAChRs using identical experimental techniques and data analysis has not been conducted.

Because of their high permeability to Ca^{2+} ions, NMDARs and α7 nAChRs form excellent examples of ligand-gated Ca^{2+} ion channels. As discussed, moderate activation of these receptors and thus, moderate elevation in $[Ca^{2+}]_i$ have been found to be neuroprotective in a number of *in vitro* and *ex vivo* toxicity models as well as *in vivo* settings [18, 21, 23, 25–27, 41–44, 114–116]. Moreover, both types of receptors appear to employ Ca^{2+}-PI3K-Akt-dependent pathways for mediation of neuroprotective effects [41–43, 49, 101, 117]. However, despite these important similarities, NMDARs and α7 nAChRs belong to different families of ligand-gated receptors [62, 118] and their kinetic and pharmacological properties are quite different. For instance, the mean open time of α7 nAChR-mediated channels (~100–400 μs, [119–121]) is at least tenfold shorter than that of NMDAR channels [63, 122]. In addition, in the continuous presence of agonist, α7 nAChR-mediated currents (but not NMDAR-mediated currents) can be completely inhibited by desensitization and/or agonist-mediated open channel block [123, 124]. The short open time and rapid desensitization act as mechanisms that protect α7 nAChR-expressing cells from excessive and thus, damaging Ca^{2+} influx. The open channel Mg^{2+} block plays an analogous role for NMDAR-mediated ion channels. By contrast, Mg^{2+} ions do not significantly alter the function of α7 nAChRs at negative membrane potentials, although they induce rectification at depolarized membrane potentials [125].

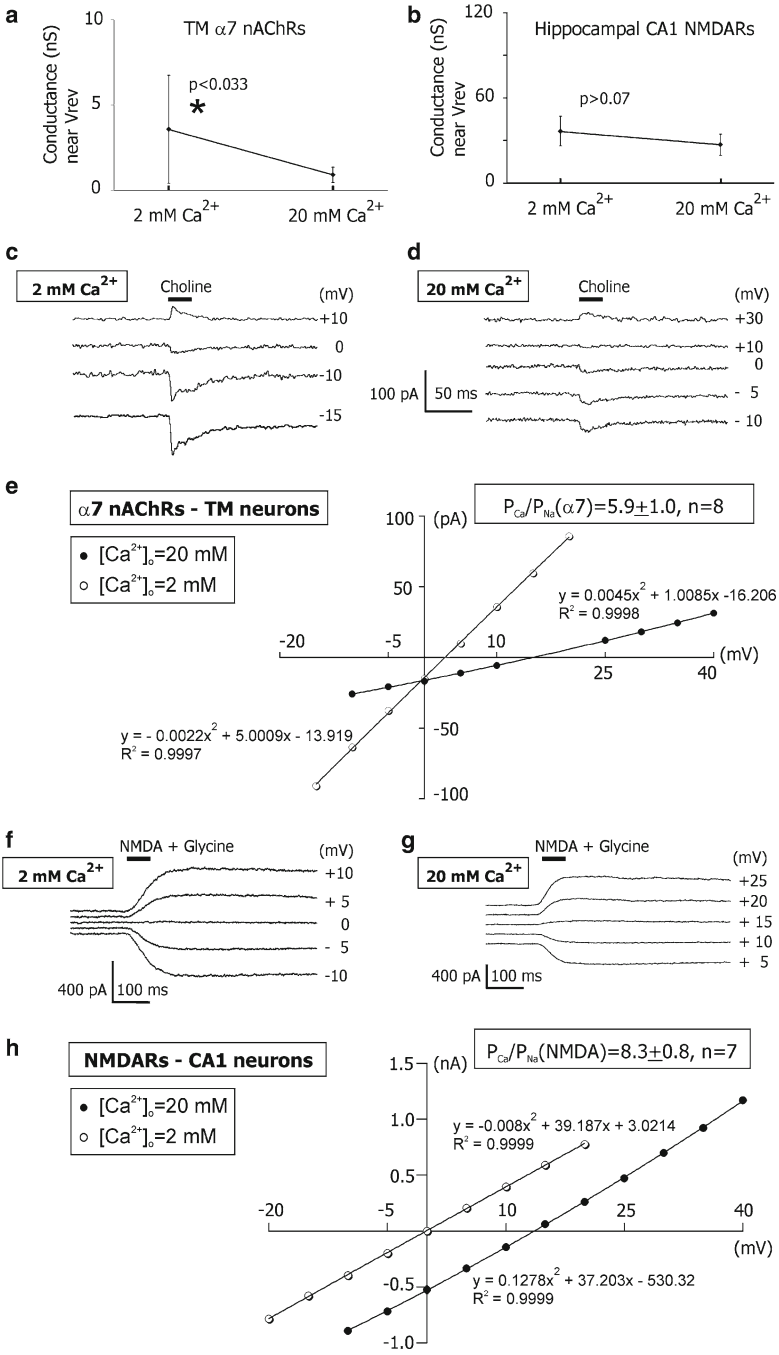
Ca^{2+} Permeability of α7 nAChRs and NMDARs

The sensitivity of α7 nAChR- and NMDAR-mediated whole-cell responses to external Ca^{2+} ions (i.e., $[Ca^{2+}]_o$) have also been found to be different (Fig. 27.1, [97]). The whole-cell conductance of α7 nAChR-mediated responses in tuberomammillary (TM) neurons was significantly greater at low $[Ca^{2+}]_o$ (i.e., 2 mM) than at high $[Ca^{2+}]_o$ (i.e., 20 mM) [97]. This difference was not due to a current rundown because experiments in low $[Ca^{2+}]_o$ that gave larger currents were conducted after experiments in high $[Ca^{2+}]_o$ that gave smaller currents [97]. By contrast, a tenfold increase in $[Ca^{2+}]_o$ from 2 to 20 mM did not significantly reduce the whole-cell conductance of NMDAR-mediated responses near their reversal potential in acutely dissociated hippocampal CA1 neurons [97]. Similar observations have been made in single-channel [126] and whole-cell [90, 127] experiments in cultured hippocampal neurons. However, a 67-fold increase in $[Ca^{2+}]_o$ from 0.3 to 20 mM has been reported to reduce the whole-cell conductance of NMDAR-mediated currents by 32% in cultured spinal cord and hippocampal neurons [128]. These differences in Ca^{2+} sensitivity of α7 nAChR- and NMDAR-mediated ion channels may reflect different affinities with which Ca^{2+} ions block monovalent permeation [129], and/or a potential Ca^{2+}-dependent modulation of α7 nAChR-channel kinetics and/or binding. All of these effects would be expected to make excessive activation of α7 nAChRs somewhat less damaging than equivalent activation of NMDARs. These views are consistent with recent experimental results [41, 43]: in these experiments, preconditioning of retinal ganglion cells in very high concentrations of nicotine (i.e., <500 μM), but not glutamate, was neuroprotective against glutamate toxicity.

In addition to Ca^{2+} permeability, the impact of activation of ligand-gated Ca^{2+} channels on cellular behavior and survival is affected by the channel distribution within the cell and the cell surface [50, 54, 130–132]. As mentioned, synaptic NMDARs promote neuroprotection, while extrasynaptic NMDARs may be neurotoxic [133]. By contrast, functional neuronal α7 nAChRs are predominantly pre- or extrasynaptic with only a handful of known exceptions [134–137] and yet, moderate activation of α7 nAChRs is usually neuroprotective. The reason for this important difference between NMDARs and α7 nAChRs is unknown and it is likely that other receptor properties (e.g., kinetic and desensitization properties) in addition to receptor location and ion channel Ca^{2+} permeability contribute to determining the receptor role in neuronal survival.

Desensitization vs. Open-Channel Block of α7 nAChRs

In the continuous presence of nicotinic agonists, activation of α7 nAChRs is reduced naturally by two independent processes: desensitization and open channel block by agonist molecules. It is important to distinguish between these processes, especially if high concentrations of agonists are used (e.g., >2 mM ACh). At negative membrane voltages, positively charged agonists (e.g., ACh, choline) elicit both desensitization


a
TM α7 nAChRs
Conductance (nS) near Vrev
10
5
0
p<0.033
*
2 mM Ca2+
20 mM Ca2+
b
Hippocampal CA1 NMDARs
120
60
0
p>0.07
c
2 mM Ca2+
Choline
(mV)
+10
0
-10
-15
d
20 mM Ca2+
+30
+10
0
- 5
- 10
100 pA
50 ms
e
α7 nAChRs - TM neurons
• [Ca2+]o=20 mM
○ [Ca2+]o=2 mM
PCa/PNa(α7)=5.9±1.0, n=8
100
(pA)
50
-50
-100
y = 0.0045x2 + 1.0085x -16.206
R2 = 0.9998
y = - 0.0022x2 + 5.0009x - 13.919
R2 = 0.9997
-20
-5
10
25
40
(mV)
f
NMDA + Glycine
+ 5
400 pA
100 ms
g
+25
+20
+ 15
+ 10
h
NMDARs - CA1 neurons
PCa/PNa(NMDA)=8.3±0.8, n=7
1.5
(nA)
1.0
0.5
-0.5
-1.0
y = -0.008x2 + 39.187x + 3.0214
R2 = 0.9999
y = 0.1278x2 + 37.203x - 530.32
R2 = 0.9999

and open channel block of α7 nAChR ion channels [123]. The desensitization component of α7 nAChR-mediated responses elicited by ACh or choline can be isolated by conducting electrophysiological experiments at positive membrane voltages [123]. At negative membrane voltages, when high agonists concentrations are used (e.g., >2 mM ACh), open channel block is nearly complete although fully reversible. To minimize open channel block at negative membrane voltages, lower agonist concentrations should be used (e.g., <200 μM ACh) because the block is low-potency. By contrast, if weakly charged agonists are used (e.g., [3-(2,4-dimethoxybenzylidene)-anabaseine, i.e., DMXBA, the code name GTS-21], $pK_a \sim 7.4$, [138]), the separation of desensitization from open channel block is more challenging as open channel block is less dependent on the membrane voltage. In these cases, low agonist concentrations (e.g., <30 μM DMXBA) need to be used to reduce the contribution of open channel block to current decay [123].

Effects of Activation and Inactivation of α7 nAChRs

While in some models of neurotoxicity high concentrations of α7 nAChR agonists caused cellular death [25]; in other models, even very high concentrations of nicotine (e.g., 500 μM) promoted neuronal survival [41]. These discrepancies in results

Fig. 27.1 The whole-cell conductances of α7 nAChR- and NMDAR-mediated responses near the reversal potential. The mean and standard deviation of the slope conductance near V_{rev} built for TM α7 nAChR- (**a**) and hippocampal CA1 pyramidal NMDAR-mediated responses (**b**). A significant $[Ca^{2+}]_o$–dependent decrease in the whole-cell conductance of TM α7 nAChR-, but not CA1 NMDAR-mediated responses was observed [97]. This decrease was not due to a current rundown because it persisted in experiments where high (i.e., 20 mM) $[Ca^{2+}]_o$ was used before low (i.e., 2 mM) $[Ca^{2+}]_o$ [97]. Examples of TM α7 nAChR-mediated currents obtained by applications of choline at various positive and negative membrane voltages in voltage-clamp in 2 mM $[Ca^{2+}]_o$ (**c**) and 20 mM $[Ca^{2+}]_o$ (**d**). The whole-cell conductance of TM α7 nAChR channels in high $[Ca^{2+}]_o$ was always lower than that in low $[Ca^{2+}]_o$, presumably due to a Ca^{2+}-dependent block of monovalent ion permeation. (**e**) The current–voltage relationship for responses illustrated in (**c**) and (**d**). No considerable current rectification was observed owing to Mg^{2+}-free external and internal solutions and the presence of F^- ions in the internal solution. The I-V curves were fitted with second-order polynomial equations. Panels (**c**–**e**) illustrate data obtained from the same acutely dissociated TM neuron. Examples of CA1 NMDAR-mediated currents obtained by applications of NMDA plus glycine at various positive and negative membrane voltages in voltage-clamp in 2 mM $[Ca^{2+}]_o$ (**f**) and 20 mM $[Ca^{2+}]_o$ (**g**). (**h**) The current–voltage relationship for responses illustrated in (**f**) and (**g**). The whole-cell conductance of NMDAR channels in 20 mM $[Ca^{2+}]_o$ was similar to that in 2 mM $[Ca^{2+}]_o$, indicating a lack of significant Ca^{2+}-dependent block of monovalent ion permeation. The I-V curves were fitted with second-order polynomial equations. Panels (**f**–**h**) illustrate data obtained from the same acutely dissociated hippocampal CA1 neuron. Note that although the application pipettes were filled with 40 mM choline or 200 μM NMDA + 20 μM glycine, the effective concentrations of choline or NMDA+glycine near the recorded neurons were unknown and considerably lower than the concentrations of agonists in application pipettes. However, in each given experiment these concentrations were very stable evidenced by stable responses [97] (Reprinted from Uteshev [97] with permission from Blackwell Publishing in the format Journal via Copyright Clearance Center)

may be linked to differences in the agonist concentration and time course of agonist application, as well as inactivation, desensitization and other kinetic properties of α7 nAChRs, e.g., open channel block by nicotinic agonists [123, 124, 139, 140]. Notably, low concentrations of nicotinic agonists such as those observed in the cerebrospinal fluid (CSF) *in vivo* (e.g., <1 μM nicotine or <100 μM choline) are more likely to cause desensitization than activation of α7 nAChRs [124, 140]. Accordingly, it has been hypothesized that it is desensitization or inhibition and not activation of α7 nAChRs that may trigger intracellular events responsible for neuroprotection and cognitive benefits [141–143]. This hypothesis, however, cannot explain a number of recent experimental findings. For instance, systemic administration of PNU-120596, a nicotinic agent that considerably reduces α7 nAChR desensitization (see below), produced positive behavioral effects restoring auditory gating deficit in a mouse model of schizophrenia [32]. Moreover, a direct testing of this hypothesis using structurally similar high-efficacy (i.e., full) and low-efficacy (i.e., partial) α7 nAChR agonists clearly demonstrated that activation of α7 nAChRs is essential for cognitive enhancement in a rat model of inhibitory avoidance [144]. Similarly, the eye-blink conditioning response is improved by α7 nAChR agonists, but impaired by antagonists [145–147] and in α7 knock-out animals [148]. Finally, cell death induced by excessive, but not moderate activity of α7 nAChRs in the NGF/serum-withdrawal toxicity model in pheochromocytoma-12 (PC-12) cells expressing functional α7 nAChRs supports the need for activation rather than desensitization of α7 nAChRs for survival of PC-12 cells [25].

By contrast, the role of α7 nAChRs in the pathophysiology of AD is less defined, primarily because of the limited understanding of how α7 nAChRs interact with $A\beta_{1\text{-}42}$. For example, both activation and blockade of α7 nAChRs inhibits $A\beta_{1\text{-}42}$-induced phosphorylation of tau proteins in PC-12 cells [143]. One hypothesis is that although activation of α7 nAChRs is neuroprotective and cognitively beneficial in some experimental models [23, 149–153], in mouse models of late stages of AD, which correlate with an excessive accumulation of $A\beta_{1\text{-}42}$, the role of α7 nAChRs reverses. The mechanism of this role reversal may include continuing high-affinity binding of $A\beta_{1\text{-}42}$ to α7 nAChRs and formation of α7-$A\beta_{1\text{-}42}$ complexes which inhibit and even reverse the physiological function of α7 nAChRs and thus, the neuroprotective binding of nicotinic agonists to α7 nAChRs becomes impaired [150, 154–161]. This hypothesis received additional support from a number of recent studies that demonstrated that blocking or eliminating α7 nAChRs could alleviate some symptoms of AD. Specifically, (1) deletion of the α7 nAChR gene ameliorates certain behavioral deficits in a transgenic mouse model of AD [162]; (2) intracellular accumulation of $A\beta_{1\text{–}42}$ that occurs predominantly in α7 nAChR-expressing neurons is blocked by α-bungarotoxin, a selective α7 nAChRs antagonist and by phenylarsine, an inhibitor of endocytosis [163]; and (3) α7 nAChRs mediate $A\beta_{1\text{-}42}$-induced phosphorylation of tau proteins [154, 155]. These experiments supported the idea of high-affinity binding of $A\beta_{1\text{–}42}$ to α7 nAChRs on neuronal cell surfaces [164], subsequent endocytosis of the resulting α7-$A\beta_{1\text{-}42}$ complex and its accumulation within the lysosomal compartment provoking intracellular toxicity [163, 165].

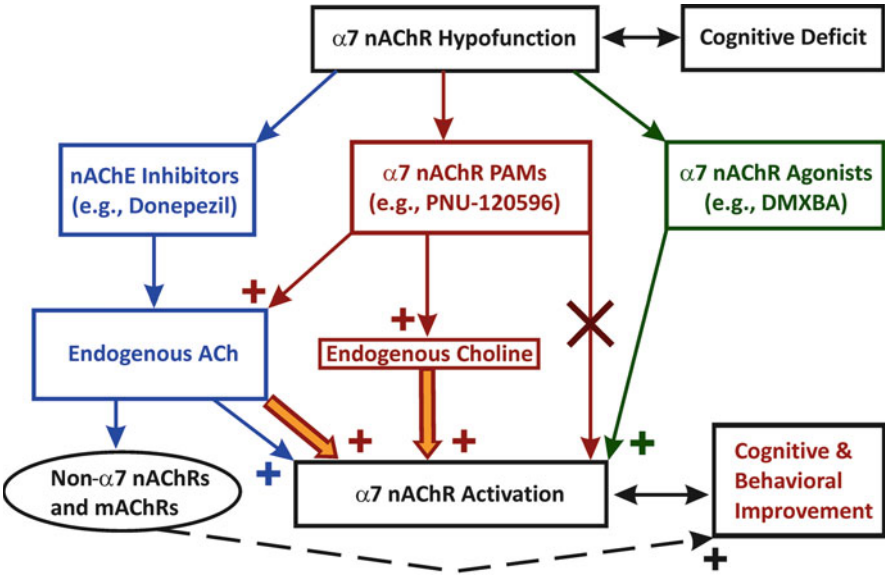


Fig. 27.2 Therapeutic approaches aimed at rescuing the brain α7 nAChR activation. The left most pathway: ACh esterase inhibitors (e.g., donepezil) increase the CSF level of ACh and promote activation of both nAChRs and mAChRs. Despite cognitive benefits (*dashed line*), the lack of selectivity may cause considerable side effects (e.g., autonomic). The right most pathway: α7 nAChR agonists. A moderate activation of α7 nAChRs by selective agonists (e.g., DMXBA) protects neurons, benefits cognition and appears to be clinically safe. The middle pathway: positive allosteric modulators (PAMs) of α7 nAChRs. Choline is a low-potency endogenous selective agonist of α7 nAChRs, but its potency can be considerably increased by Type-II α7-PAMs, such as PNU-120596. α7-PAMs do not activate α7 nAChRs in the absence of nicotinic agonists. Instead, α7-PAMs lower the energy barrier, allowing lower concentrations of nicotinic agonists to activate the receptor. In the presence of Type-II α7-PAMs, endogenous choline may become effective in producing moderate persistent activation of native α7 nAChRs. This type of activation of α7 nAChRs may promote neuroprotection and benefit cognition

α7 nAChRs as a Therapeutic Tool

There is a substantial body of supportive evidence linking age-, disease- and trauma-related alterations in the expression and function of α7 nAChRs to neurodegenerative, sensorimotor and psychiatric disorders associated with cognitive decline and attention deficits [101, 166–180]. By contrast, activation of α7 nAChRs by nicotine and selective α7 nAChR agents has been shown to produce neuroprotection *in vivo* [26, 150, 181], *ex vivo* and *in vitro* [18, 21, 23, 25–27, 182–189] and enhance cognitive performance in patients and animal models of neurodegenerative disorders including AD, schizophrenia, brain trauma and aging [32, 101, 148, 181, 183, 189–209].

Deficits in hippocampal α7 nAChR activation are a key accompanying factor in certain cognitive disorders and enhancing this activation by nicotinic agonists has been shown to produce neuroprotection and cognitive benefits. Currently available therapeutic approaches aimed at rescuing the brain α7 nAChR activation include (Fig. 27.2): (1) ACh esterase inhibitors (AChE; e.g., donepezil) – the left most

pathway; (2) α7 nAChR agonists – the right most pathway; and (3) positive allosteric modulators (PAMs) of α7 nAChRs – the middle pathway. The rationale for therapeutic use of α7 nAChR agonists and modulators arrives from observations that in neurological disorders such as dementia and schizophrenia as well as after brain trauma, functional α7 nAChRs expressed in central neurons do not vanish but their number may decline in a region-specific manner [167, 168, 171, 173, 177, 178, 180, 210]. Therefore, a moderately enhanced activation of α7 nAChRs can be achieved by pharmacological tools and this enhancement may benefit patients with neurodegeneration and cognitive decline (see Sects. 3.1, 3.2, 3.3, 3.4, 3.5).

Positive cognitive effects of inhibitors of AChE result from inhibition of the hydrolysis of ACh and thus, enhanced activation of both muscarinic AChRs (i.e., mAChRs) and nAChRs, including α7 subtype (Fig. 27.2, the left most pathway). Similar to α7 nAChRs, activation of mAChRs and non-α7 nAChRs has been reported to be cognitively beneficial (horizontal dashed path, Fig. 27.2) [211–217]. However, the lack of specificity may cause autonomic adverse effects. For example, donepezil and other AChE inhibitors have been reported to cause centrally-mediated nausea, vomiting and diarrhea [218, 219].

As discussed earlier, a moderate activation of α7 nAChRs by selective agonists (e.g., DMXBA, the right most pathway, Fig. 27.2) protects neurons, benefits cognition and appears to be clinically safe. For example, no major central side effects have been linked to oral administration of large doses of DMXBA (e.g., <450 mg/day, [138, 192]). In hippocampal slices, activation of α7 nAChRs by therapeutic nicotinic agonists, such as DMXBA, can be potentiated by PAMs [220]. PAMs would also be expected to enhance activation of α7 nAChRs by physiological levels of endogenous nicotinic agonists (i.e., ACh and choline) [34, 35] released naturally as needed.

Effects of PAMs on α7 nAChR Activation and Ca^{2+} Influx

PAM Hypothesis

Choline is an endogenous selective agonist of α7 nAChRs [221, 222]. The cerebrospinal fluid (CSF) contains choline at concentrations much lower (~5–10 μM, [169, 223–227]) than its EC_{50} (~0.5–1.5 mM; [222, 228]). Moreover, choline exhibits a much greater potency for desensitization (IC_{50} ~ 40 μM, [124]) than activation of α7 nAChRs. Therefore, the endogenous concentration of choline in the CSF appears to be too low to activate α7 nAChRs [34, 35, 124] and in the past, endogenous choline has not been seriously considered as a therapeutic candidate [186]. However, the ambient levels of choline can be elevated 3–4-fold under conditions associated with ischemia, stroke, and substantial plasma membrane damage [223, 224, 226, 227, 229]. Cell death also creates a large source of choline causing a breakdown of phosphatidylcholine, the principle plasma membrane phospholipid, into choline and diacylglycerol. Given the low ambient concentrations of choline

in the CSF under physiological conditions [169, 225], it is unlikely that in the absence of cholinergic synaptic inputs or exogenous nicotinic agents, native α7 nAChRs are persistently activated by endogenous choline [124]. However, the effects of endogenous choline may be notably different in the presence of Type-II α7-PAMs, such as PNU-120596, which significantly enhances the responsiveness of α7 nAChRs to nicotinic agents (see Sects. 3.2, 3.3, 3.4). PNU-120596 is a positive allosteric modulator of α7 nAChRs that reduces desensitization of α7 nAChRs and thus, increases the potency of nicotinic agonists enhancing the responsiveness of functional α7 nAChRs [32, 34, 220, 230, 231] and producing behavioral improvements in animal models [32]. PNU-120596 has been shown to increase the mean open time of α7 nAChR channels without producing significant changes in ion channel selectivity, single channel conductance and Ca^{2+} permeability [32]. PNU-120596 does not activate α7 nAChRs in the absence of nicotinic agonists. Instead, it lowers the energy barrier, allowing lower concentrations of nicotinic agonists to activate the receptor [232]. Intravenous administration of 1 mg/kg PNU-120596 elevates the concentration of PNU-120596 in the brains of rats to ~1.5 μM [32]. This value falls near the EC_{50} for potentiating effects of PNU-120596 (EC_{50} ~ 1.5 μM) [233, 234]. Concentrations slightly lower than the EC_{50} (i.e., 1 μM PNU-120596) have been shown to enhance the effects of sub-threshold concentrations of choline allowing physiological levels of choline to become effective in activation of native α7 nAChRs in the absence of exogenous nicotinic agents [34, 35]. Therefore, in the presence of PNU-120596, endogenous choline may become effective in producing moderate persistent activation of α7 nAChRs and the corresponding elevation in the Ca^{2+} influx and neuronal excitability (see Sects. 3.3 and 3.4) supporting neuroprotection and cognition (see Sect. 2.5).

There are two types of PAMs [235]: Type I – these compounds enhance the amplitude of α7 nAChR-mediated currents without affecting the current duration; and Type II – these compounds dramatically reduce desensitization and thus, prolong the duration of activation of α7 nAChRs in the constant presence of agonists (Fig. 27.3). The Type-II PAMs (e.g., PNU-120596) are most interesting because these compounds not only reduce desensitization of α7 nAChRs but also allow nicotinic agonists to activate already desensitized α7 nAChRs [32]. Therefore, in the presence of Type-II α7-PAMs, desensitization does not contribute to α7 nAChR activation deficits and previously desensitized α7 nAChRs can be successfully recruited for activation. Recent studies have also demonstrated that PNU-120596 is able to increase the activation potency of choline, allowing low sub-threshold (for activation) physiological concentrations of choline (~10 μM) to become effective in activation of α7 nAChRs [34, 35]. This finding suggests an intriguing possibility of using endogenous choline (in the presence of Type-II α7-PAMs) as a therapeutic agent for enhancing activation of α7 nAChRs and thus, Ca^{2+} influx in neuronal systems characterized by cholinergic deficiency.

A reduced version of this hypothesis has been tested in *ex vivo* electrophysiological experiments using hypothalamic and hippocampal brain slices [34, 35]. Under this scenario, endogenous levels of choline were modeled by the addition of physiological concentrations of choline (5–10 μM) to artificial cerebrospinal solution

(ACSF) and whole-cell voltage- and current-clamp recordings were conducted in the presence and absence of 1–5 μM PNU-120596 to determine the effects of enhanced activation of native α7 nAChRs by choline on the electrical activity of hypothalamic and hippocampal neurons in brain slices (Figs. 27.4 and 27.5).

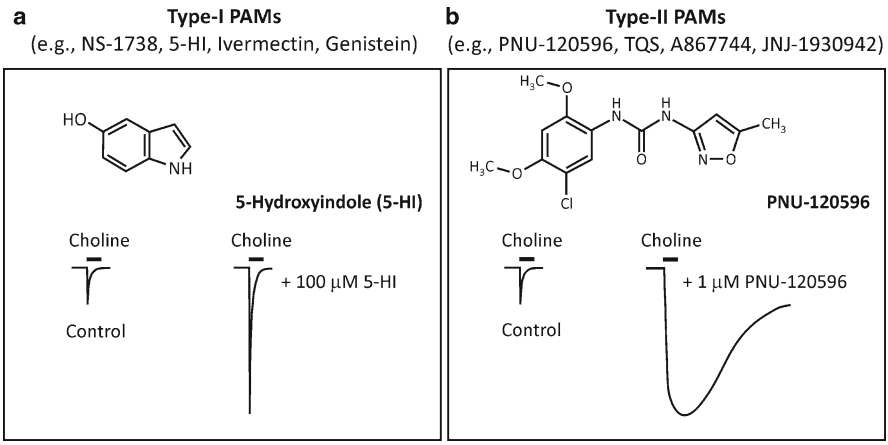
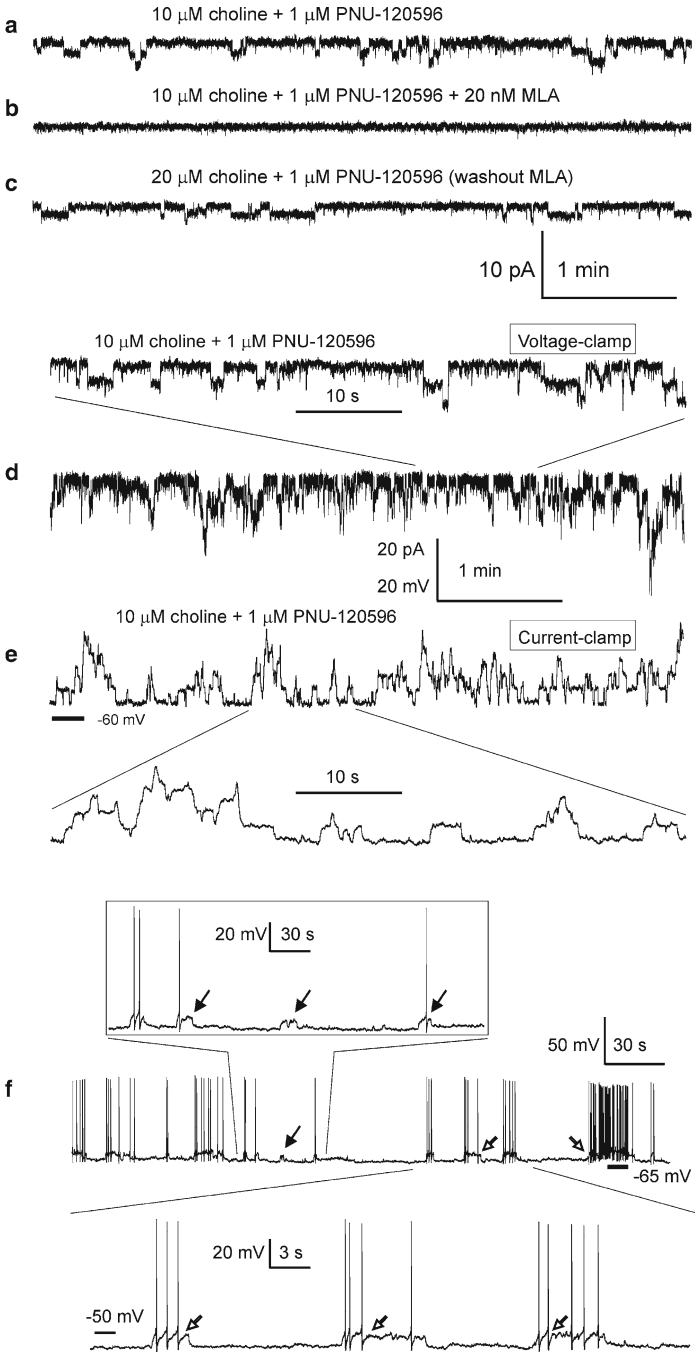
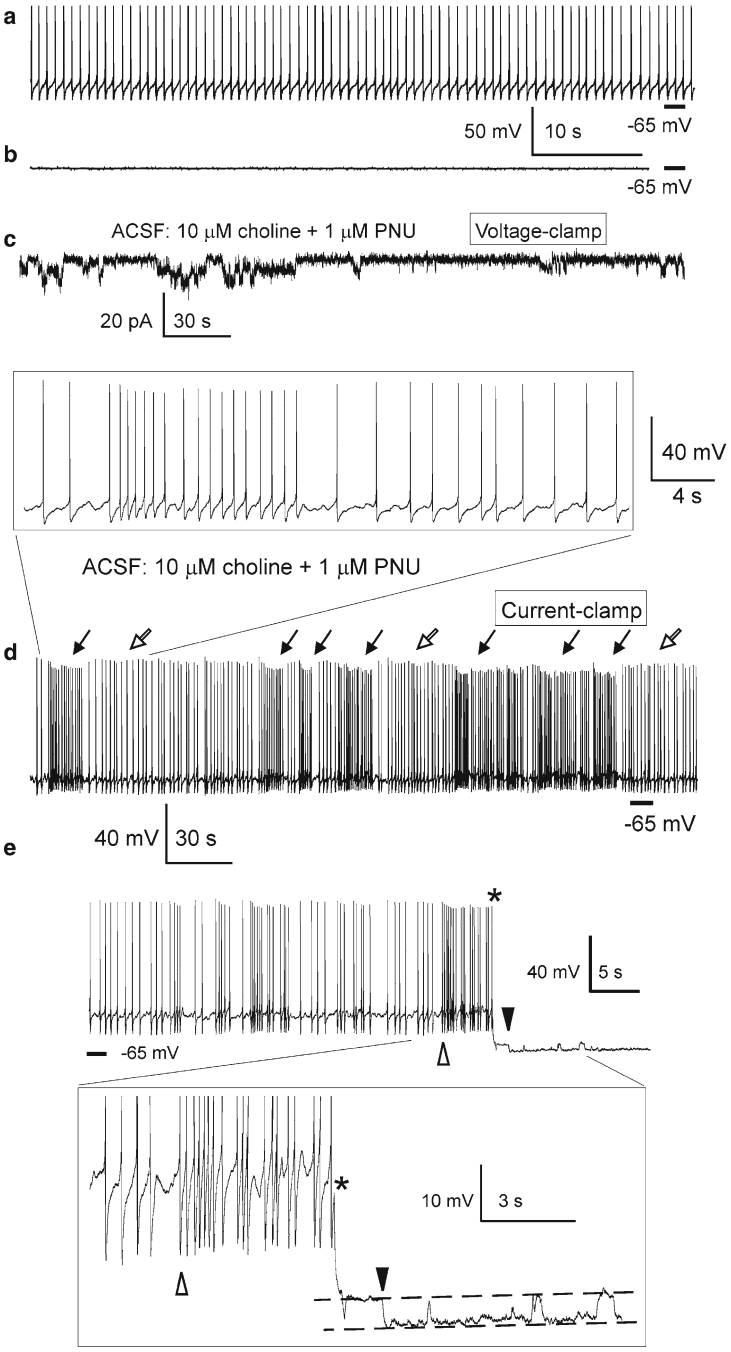


Fig. 27.3 Examples and illustrative effects of Type-I and Type-II α7-PAMs. (**a**) NS-1738, 5-HI, Invermectin and Genistein represent the family of Type-I α7-PAMs. Schematic current traces illustrate the effects of Type-I α7-PAMs on α7 nAChRs: Type-I α7-PAMs increase the peak of α7 nAChR-mediated responses but do not alter the rate of desensitization of α7 nAChRs. (**b**) PNU-120596, TQS, A867744, JNJ-1930942 represent the family of Type-II α7-PAMs. Schematic current traces illustrate the effects of Type-II α7-PAMs on α7 nAChRs: Type-II α7-PAMs increase the peak of α7 nAChR-mediated responses and considerably reduce the desensitization of α7 nAChRs

Fig. 27.4 Step-like current and voltage deviations in the presence of 10 μM choline and 1 μM PNU-120596 in ACSF. (**a**–**c**) Current deviations were completely and reversibly blocked by 20 nM MLA, confirming the involvement α7 nAChRs. All current traces in (**a**–**c**) were obtained from the same TM neuron. (**d, e**) Step-like responses were observed in both voltage- (**d**) and current-clamp (**e**) recordings. Traces in (**d**) and (**e**) were obtained from the same TM neuron 1 min apart. In these experiments, the frequency of step-like current events appeared to be sensitive and rapidly responsive to changes in the ACSF concentrations of choline and PNU-120596 [34, 35]. Activation of α7 nAChRs in current-clamp elicited transient repetitive step-like depolarizations: ~4 mV for individual events and ~25 mV for simultaneous multiple events (**e**). The bottom trace in (**d**) and the top trace in (**e**) share the same time scale shown between these traces. The vertical scale bar indicates either 20 pA (for traces in **d**) or 20 mV (for traces in **e**). In experiments shown in (**d, e**), 0.3 μM TTX was continuously present in ACSF and the internal pipette solution contained $CsMeSO_3$. In voltage-clamp experiments, the membrane voltage was held at −60 mV. (**f**) To visualize individual step-like depolarizations, a small continuous hyperpolarizing current (−5 pA) was injected into the recorded neuron resulting in cessation of spontaneous firing. Under these silent conditions, transient step-like depolarizations triggered short trains of action potentials (*open arrows*). However, occasionally, depolarizations did not trigger action potentials or triggered only a single action potential per depolarization (*filled arrows*). Step-like voltage and current deviations were resistant to 20 μM gabazine, 15 μM DNQX, 50 μM AP-5, 40 μM picrotoxin, and 0.3 μM TTX applied to ACSF (Reprinted from Gusev and Uteshev [34] with permission from ASPET)


a
10 μM choline + 1 μM PNU-120596
b
10 μM choline + 1 μM PNU-120596 + 20 nM MLA
c
20 μM choline + 1 μM PNU-120596 (washout MLA)
10 pA
1 min
10 μM choline + 1 μM PNU-120596
Voltage-clamp
10 s
d
20 pA
20 mV
1 min
10 μM choline + 1 μM PNU-120596
Current-clamp
e
-60 mV
10 s
20 mV
30 s
50 mV
30 s
f
-65 mV
20 mV
3 s
-50 mV


a
50 mV
10 s
-65 mV
b
-65 mV
c
ACSF: 10 μM choline + 1 μM PNU
Voltage-clamp
20 pA
30 s
40 mV
4 s
ACSF: 10 μM choline + 1 μM PNU
Current-clamp
d
-65 mV
e
40 mV
30 s
40 mV
5 s
-65 mV
10 mV
3 s

Synergistic Action of Physiological Choline and PNU-120596

Intriguingly, current and voltage deviations recorded in voltage- and current-clamp, respectively, resulting from a synergistic action of 10 μM choline plus 1–2 μM PNU-120596 were step-like and thus, reminiscent of and postulated to be single α7 nAChR ion channel openings detectable in whole-cell patch-clamp configuration (Fig. 27.4a–e). These experiments revealed that in the presence of PNU-120596 and 5–10 μM choline, even very low densities of α7 nAChRs such as the expression found in hippocampal CA1 pyramidal neurons (only ~5% of that found in hippocampal CA1 interneurons [35]) generate persistent step-like currents which cause transient step-like depolarizations and occasionally, trigger bursts of action potentials. This persistent current would be expected to generate a persistent Ca^{2+} influx (see Sects. 3.4 and 3.5). A similar activity was detected under slightly hyperpolarized conditions in hypothalamic TM neurons (Fig. 27.4f). Moreover, activation of TM α7 nAChRs by 10 μM choline plus 1 μM PNU-120596 enhances spontaneous firing of TM neurons (Fig. 27.5a–d). In current-clamp, when a hyperpolarizing current (~ −40 pA) was injected in the recorded TM neuron (the injection time is marked by * (Fig. 27.5e)) during a prolonged interval of increased frequency (the interval between open and filled triangles), it resulted in cessation of spontaneous firing, allowing detection of the final portion of an underlying step-like depolarization. Therefore, a prolonged step-like depolarization was observed as an increase in

Fig. 27.5 Activation of TM α7 nAChRs by 10 μM choline plus 1 μM PNU-120596 enhances spontaneous firing of TM neurons in current-clamp. The spontaneous firing of TM neurons was native as current injections were not applied (i.e., 0 pA). Horizontal bars indicate −65 mV. In current-clamp, in the absence of PNU-120596 and choline, TM neurons exhibited regular patterns of spontaneous firing (**a**). In these control experiments, when the membrane voltage was hyperpolarized to −65 mV by injections of a small current, step-like depolarizations were not observed (**b**). Recordings in (**a**) and (**b**) were obtained from the same TM neuron 1 min apart. After the sustained repetitive activation of TM nAChRs was observed in voltage-clamp upon administration of 10 μM choline plus 1 μM PNU-120596 (**c**), current-clamp recordings were conducted using the same TM neuron (**d**). In current clamp, activation of TM α7 nAChRs resulted in transient repetitive increases in the frequency of spontaneous firing of TM neurons (**d**, *filled arrows*). Traces shown in (**c**) and (**d**) were obtained from the same TM neuron 1 min apart. The framed insert in (**d**) illustrates at a higher time resolution a portion of recording containing one transient excitation. (**e**) The effects of individual step-like depolarizations in current clamp. When a hyperpolarizing current (~ −40 pA) was injected in the recorded TM neuron (the injection time is marked by *) during a prolonged interval of increased frequency (the interval between *open* and *filled triangles*), it resulted in cessation of spontaneous firing, allowing detection of the final portion of an underlying step-like depolarization. Therefore, a prolonged depolarization was observed as both an increase in spontaneous firing in the beginning of depolarization (*open triangle*) and a depolarizing step at the end of depolarization (*filled triangle*). Subsequent step-like depolarizations are also seen between the two dashed lines in insert. The insert illustrates this transition process at a higher resolution. In these experiments, ACSF contained 20 μM, gabazine, 15 μM DNQX, 50 μM AP-5 and 40 μM picrotoxin. The internal solution was K-gluconate-based (Reprinted from Gusev and Uteshev [34]. With permission from ASPET)

spontaneous firing in the beginning of depolarization (Fig. 27.5e, open triangle) and a depolarizing step at the end of depolarization (Fig. 27.5e, filled triangle).

In these experiments, the frequency of step-like current events appeared to be sensitive and rapidly responsive to changes in the ACSF concentrations of choline and PNU-120596 [34, 35]. Therefore, the synergistic action of endogenous choline and Type-II α7-PAMs may cause a sustained activation of α7 nAChRs and the corresponding persistent Ca^{2+} influx (see Sects. 3.4 and 3.5). These observations suggest that the net depolarization, excitation and Ca^{2+} influx could be modulated and optimized by tuning the administration doses of dietary choline [189] and Type-II α7-PAMs [34, 35].

Detection of Activity of Individual α7 nAChRs in Whole-Cell

It is this capability of as few as only one individual functional α7 nAChR to depolarize and excite the entire neuron that makes it possible for a low density expression of functional α7 nAChRs to be effective in enhancing the excitability of hippocampal CA1 pyramidal neurons in the presence of PNU-120596 [35]. Therefore, high levels of expression of α7 nAChRs and synchronization of their activity may not be required for significant depolarizing and excitatory effects of physiological concentrations of choline in the presence of PNU-120596. The excitability of hippocampal CA1 pyramidal neurons positively correlate with cognitive performance and has been shown to decline with age likely due to an age-dependent enhancement of inhibitory effects of the Ca^{2+}-dependent potassium conductance [236, 237]. Therefore, therapeutic approaches that provide neuroprotection and restore excitability of hippocampal CA1 pyramidal neurons may benefit patients with various forms of dementia and brain trauma.

Detecting activity of individual α7 nAChR ion channels in whole-cell patch-clamp experiments appears to be possible if the probability of ion channel openings is sufficiently low and the channels remain open for a prolonged period of time during which the ionic gradient across the membrane and thus, the ionic current, remain relatively constant. These requirements appear to be fulfilled for α7 nAChRs activated by physiological concentrations of choline in the presence of 1–5 μM PNU-120596 in hippocampal CA1 pyramidal neurons [35], hippocampal CA1 interneurons (Kalappa and Uteshev, unpublished observations) and hypothalamic TM α7 nAChRs [34].

In current-clamp patch-clamp experiments using hippocampal CA1 pyramidal neurons that express a very low density of functional α7 nAChRs [35], individual step-like voltage deviations triggered action potentials in 7 out of 13 cells tested (Fig. 27.4b, c). When these deviations failed to cause action potentials, they generated small step-like depolarizations whose amplitudes (~3–5 mV) could be predicted from the neuronal input resistance (~500 MΩ), the amplitude of step-like currents (~8 pA) and the Ohm's law (500 MΩ × 8 pA ~4 mV). These estimates support the hypothesis that the observed single channel openings were most likely

generated by α7 nAChRs expressed in both proximal and distal regions of the neuronal membrane and not generated only by α7 nAChRs located in the immediate vicinity of the recording patch electrode. An additional support to this hypothesis comes from the observation that in current-clamp experiments with hippocampal CA1 pyramidal neurons, recorded action potentials were triggered by α7 nAChR-mediated step-like depolarizations, while action potentials in between step-like depolarizations were not detected [35]. Therefore, it is unlikely that step-like depolarizations generated by distal α7 nAChRs (e.g., located far away from the recording pipette) have been routinely undetected (due to, for example, electrotonic filtering) because action potentials generated by distal α7 nAChRs would have occurred randomly including in between detected step-like depolarizations and this has not been observed.

These findings support the hypothesis that in the presence of PNU-120596, whole-cell patch-clamp recordings are able to detect α7 nAChR-mediated single ion channel openings from the entire cell surface. This conclusion justifies use of this approach for estimation of the total whole-cell influx of Ca^{2+} ions (see Sect. 3.4).

Current Net Charge and Ca^{2+} Influx

The mean net charge per min generated by hippocampal CA1 pyramidal α7 nAChR ion channels in response to 10 μM choline plus 2 μM PNU-120596 was estimated to be ~9.3 pC/min = 0.16 pA [35]. This value is nearly tenfold smaller than the mean net charge of TM α7 nAChR-mediated responses elicited by 10 μM choline plus 1 μM PNU-120596 which was estimated to be ~84 pC/min = 1.4 pA [34]. Therefore, given the 10% fractional Ca^{2+} current, Ca^{2+} ions would be expected to enter hippocampal and TM neurons at a rate of ~0.93 pC/min and ~8.4 pC/min, respectively, which translates into a sustained Ca^{2+} current ~0.016 pA and ~0.14 pA, respectively. These Ca^{2+} currents were elicited by physiological concentrations of choline and concentrations of PNU-120596 that restored the auditory gating deficit in mice [32]. Therefore, it is reasonable to expect that in *in vivo* settings, similar rates of Ca^{2+} entry in neurons expressing very low (such as hippocampal CA1 pyramidal neurons) and very high (such as hypothalamic TM neurons) densities of functional α7 nAChRs would contribute to behavioral improvements. However, a prolonged exposure of neurons to nicotinic agonists in the presence of Type-II α7-PAMs may be cytotoxic because of excessive accumulation of Ca^{2+} in the cytosol and possible activation of Ca^{2+}-dependent apoptotic pathways (see Sects. 1.1 and 1.2).

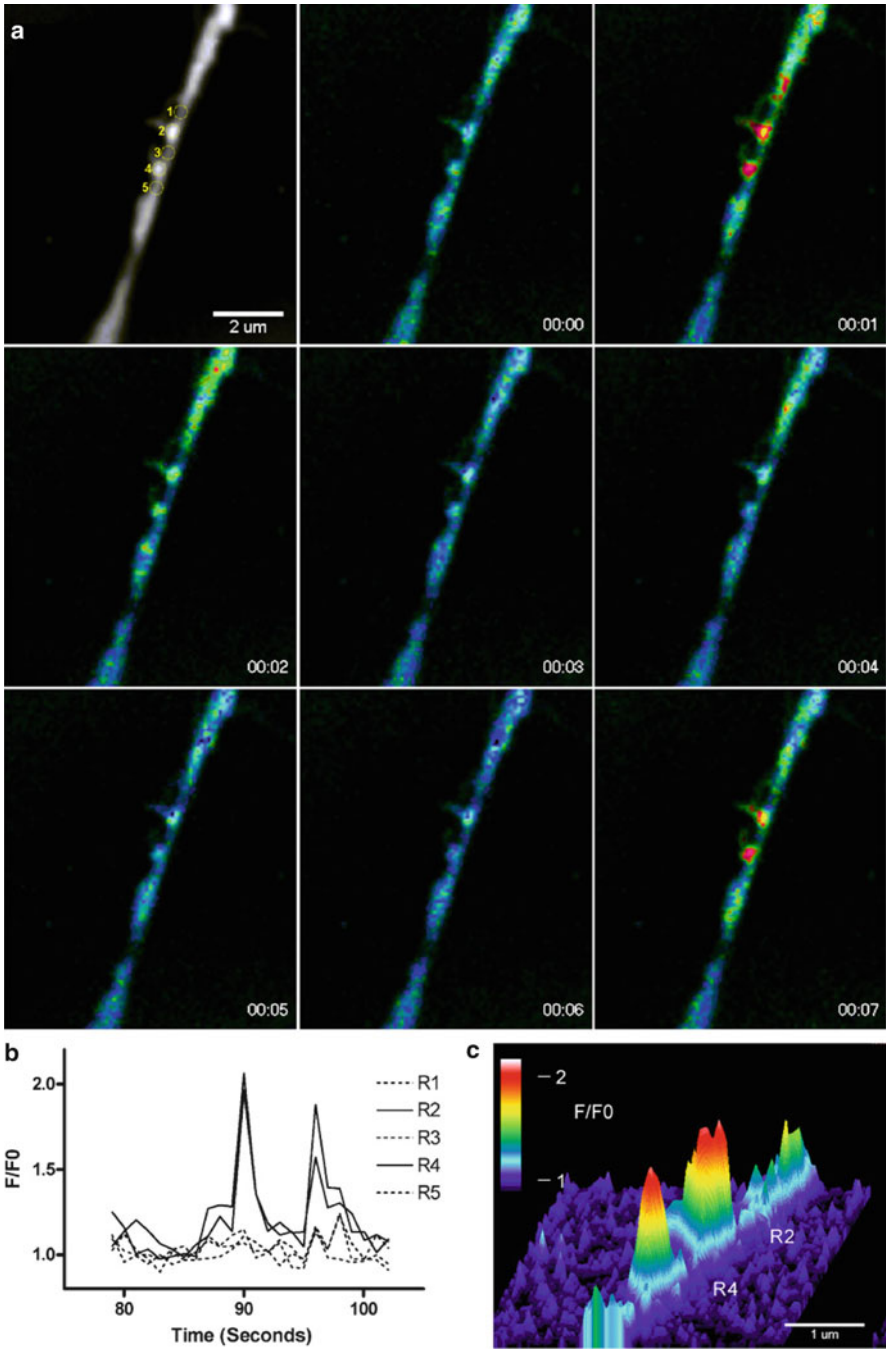
The mean number of α7 nAChR ion channels opened in hippocampal CA1 pyramidal and hypothalamic TM neurons at any given time were estimated to be $N_{pyr}P_{open} \sim 0.029$ (i.e., 0.16 pA/5.5 pA) and $N_{TM}P_{open} \sim 0.27$ (i.e., 1.4 pA/5.1 pA), respectively, where N_{pyr} and N_{TM} are the total number of detectable functional α7 nAChRs in a pyramidal and TM neuron, respectively. Note that in experiments with TM neurons, 10 μM choline plus 1 μM PNU-120596 were used [34], whereas in the

hippocampal study, the concentration of PNU-120596 was increased to 2 μM because of the substantially lower levels of expression of functional α7 nAChRs in hippocampal CA1 pyramidal neurons compared to TM neurons [35].

Direct Measurements of α7 nAChR-Mediated Ca^{2+} Influx in the Presence of PNU-120596

Openings of individual α7 nAChR-mediated ion channels recorded in whole-cell configuration would be expected to produce transient focal entries of Ca^{2+} ions. These near-membrane Ca^{2+} blinks have indeed been observed in fluorescent Ca^{2+} imaging experiments conducted in filopodia of human neuroblastoma SH-SY5Y cells and in chick retinal ganglion cells expressing α7-nAChR [238]. In the presence of PNU-120596, activation of individual and/or clusters of α7 nAChRs by nicotine resulted in transient and very focal elevations of $[Ca^{2+}]_i$ (Fig. 27.6). These Ca^{2+} blinks lasted for a few seconds and were clearly observed in the presence and absence of PNU-120596, but in the presence of PNU-120596, the frequency and the duration of Ca^{2+} blinks were considerably increased [238]. The Ca^{2+} blinks were resistant to hyperpolarization induced by valinomycin (a K^+ ionophore), but vanished upon removal of external Ca^{2+} [238]. Ryanodine (1 μM) failed to inhibit the Ca^{2+} blinks indicating that Ca^{2+} ions do not enter cells from ryanodine-sensitive cytosolic Ca^{2+} stores [238]. Figure 27.6 illustrates that, although the location and amplitudes of the Ca^{2+} blinks were variable in the presence of PNU-120596, spatiotemporally discrete Ca^{2+} blinks could be clearly resolved in the same filopodia during nicotine application. While certain distinct regions (#2 and #4) produced repetitive Ca^{2+} blinks, neighboring regions (#1, #3, and #5) did not display any Ca^{2+} events (Fig. 27.6a, b). The regions of brief Ca^{2+} elevations were localized to a submicron dimension (Fig. 27.6c). These observations further support the novel concept (see Sects. 3.1–3.4) that in the presence of Type-II α7-PAMs, individual functional α7 nAChRs generate distinct current events that may affect the behavior of the entire neuron [34, 35, 238].

Fig. 27.6 The spatiotemporal profile of the unitary Ca^{2+} events ("blinks"). (**a**) Sequential images from a time series showing two Ca^{2+} blinks separated by 1.1 μm in a single filopodia. Top left image shows the regions used for measurements overlaid on the fluorescence image, subsequent F/F0 images were captured every second during application of nicotine + PNU-120596. (**b**) Time-course of the F/F0 in two regions (#2 and #4) that exhibit repetitive Ca^{2+} elevations lasting ~3 s and in contiguous regions (#1, #3, and #5) that did not display considerable Ca^{2+} activity. (**c**) Intensity profile of the F/F0 signal at $t = 1$ s in regions #2 and #4, showing the spatial spread of the Ca^{2+} elevations. The cross-section at >20% of the peak fluorescence averaged 0.67 μm and 0.64 μm for regions #1 and #2, respectively. Cell calcium by CHURCHILL LIVINGSTONE (Reproduced from (Gilbert et al., 2009) [238] with permission of CHURCHILL LIVINGSTONE in the format Journal via Copyright Clearance Center)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Non-neuronal NMDARs and α7 nAChRs

In addition to being broadly expressed in the central and peripheral nervous systems of mammals, functional NMDARs and α7 nAChRs are expressed in the immune system [186, 239–250], cancer cells [251–257] and other non-neuronal cells that promote angiogenesis and proliferation of cancer. Activation of α7 nAChRs in non-neuronal systems inhibits inflammation and promotes development of cancer. Although the exact role of NMDARs and α7 nAChRs in immune and cancer cells is not well understood, the high permeability of these receptor ion channels to Ca^{2+} ions suggest important implications for cellular function, survival and proliferation. Therefore, activation, inhibition and modulation of NMDARs and α7 nAChRs in immune and cancer cells can be used for therapeutic purposes to regulate immune defense mechanisms, reduce inflammation, inhibit proliferation or induce apoptosis of cancer cells.

Conclusions and Future Directions

In central neurons, there appear to be multiple ways of achieving optimal levels of Ca^{2+} entrance and $[Ca^{2+}]_i$ to support neuronal function and survival. Among these are inhibition of excessive Ca^{2+} influx through NMDAR channels by low-potency use-dependent blockers, such as memantine, and enhancement of deficient Ca^{2+} influx through α7 nAChR channels by partial agonists of α7 nAChRs, such as DMXBA. Moderate activation of highly Ca^{2+}-permeable NMDAR- and α7 nAChR-mediated ion channels has been shown to support neuronal function and is crucial for neuronal survival. Recently, positive allosteric modulators (PAMs) of α7 nAChRs have been identified as a promising pharmacological tool that can be used to enhance deficient activation of α7 nAChRs associated with certain neurodegenerative disorders. α7-PAMs do not activate α7 nAChRs and thus, α7 nAChRs are activated by endogenous cholinergic agonists released naturally as needed. Activation of functional α7 nAChRs is neuroprotective and thus, beneficial to neurons that express these receptors. Although some neurons that experience age- or trauma-related deficits in excitability (e.g., hippocampal CA1 pyramidal neurons [236, 237, 258]) express only very low densities of functional α7 nAChRs [35], in the presence of Type-II α7-PAMs, these neurons may also become eligible for benefits from expression and activation of functional α7 nAChRs [35].

Recent experimental results indicated that Type-II α7-PAMs may convert endogenous choline and ACh into efficacious therapeutic agents by enhancing their potency for activation of α7 nAChRs. Therefore, in the presence of Type-II PAMs, such as 1 mg/kg PNU-120596, endogenous choline may produce moderate persistent activation of α7 nAChRs and thus, moderately enhance Ca^{2+} influx and neuronal excitability in the absence of exogenous nicotinic agonists – effects that in *in vivo* settings may produce neuroprotection and cognitive benefits. Treatments involving endogenous choline may be safer than those involving synthetic α7

nAChR agonists. Hypothetically, activation of α7 nAChRs by endogenous nicotinic agonists can be moderately enhanced by optimal doses of α7-PAMs and a balanced choline diet [189]. Ideally, α7-PAM-based therapeutic interventions should be able to deliver neuroprotective and cognitive benefits by optimizing activation of α7 nAChRs and α7 nAChR-mediated Ca^{2+} influx in neuronal systems characterized by deficient activation of α7 nAChRs. In addition, an intriguing possibility exists for α7-PAMs to join a cohort of projected drug candidates for enhancement of cognition in healthy individuals [259].

Interestingly, only ~10% of hippocampal α7 proteins are surface-expressed [132] and therefore, the CA1 hippocampal region may contain a large pool of unused α7 proteins. It is intriguing to speculate that under certain physiological conditions, this pool of dormant α7 proteins could be recruited to become functional and cell surface-expressed. It is also reasonable to expect that certain endogenous compounds could enhance α7 nAChR activity in a manner similar to α7-PAMs. Finding these conditions and mechanisms of regulation of α7 nAChR surface expression and function may have a very positive impact on the future of cholinergic therapies aimed at restoring and boosting cognition in dementia patients and healthy individuals.

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