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Review

Regulation of nicotinic acetylcholine receptors by tyrosine kinases in the peripheral and central nervous system: same players, different roles

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Abstract. Nicotinic acetylcholine receptors (nAChRs) exist in many subtypes and are found in the peripheral and central nervous system where they mediate or modulate synaptic transmission. We review how tyrosine phosphorylation and kinases regulate muscle and neuronal nAChRs. Interestingly, although some of the same kinase players interact with the various receptor subtypes, the functional consequences are different. While concerted action of MuSK, Abl- and Src-family kinases (SFKs) regulates the synaptic distribution of nAChRs at the neuromuscular junction, SFKs activate heteromeric neuronal nAChRs in adrenal chromaffin cells, thereby enhancing catecholamine secretion. In contrast, the activity of homomeric neuronal nAChRs, as found in the hippocampus, is negatively regulated by tyrosine phosphorylation and SFKs. It appears that tyrosine kinases provide the means to regulate all nAChRs; but the functional consequences, even those caused by the same kinase family, are specific for each receptor subtype and location.

Keywords. Nicotinic acetylcholine receptor, neuromuscular junction, chromaffin cells, hippocampus, tyrosine phosphorylation, Src, Abl, MuSK.

Introduction

Nicotinic acetylcholine receptors (nAChRs) are a family of ligand-gated channels that provide a cation-selective pathway across the plasma membrane. The receptors are pentamers activated by the agonists acetylcholine and nicotine, and are made up from a portfolio of different subunits [1–3]. Seventeen genes have been identified up to date, with subunits $\alpha1$, $\beta1$, γ, δ and ε building muscle-type receptors found at high density in the postsynaptic membrane of the neuromuscular junction (NMJ) [4, 5]. At the NMJ, these nAChRs are crucial in communication from brain to muscle: they mediate synaptic transmission by triggering endplate potentials that lead to action potentials and contraction of skeletal muscle fibers.

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$\alpha2$–$\alpha10$ and $\beta2$–$\beta4$ subunits, homologous to their respective muscle counterparts, form nAChRs in many different combinations found throughout the peripheral (PNS) and central (CNS) nervous systems [4]. Not surprisingly, the functions, structures and roles in pathologies of these so-called neuronal nAChRs are also very diverse [6, 7]. Major heteromeric receptors are $\alpha3\alpha5\beta4$ or $\alpha4\beta2$ combinations and these can function – in analogy to muscle nAChRs – as postsynaptic receptors mediating rapid cholinergic synaptic transmission. This process is prominent in autonomic ganglionic neurons [8] and target organs such as adrenal chromaffin cells [9], where $\alpha3\alpha5\beta4$ nAChRs mediate cholinergic input, in the latter case leading to secretion of catecholamines.

In the brain, fast synaptic transmission mediated by postsynaptic nAChRs is rare [4]. An example is the reward pathway: in the ventral tegmental area, postsynaptic $\alpha4\beta2$

nAChRs reside on GABAergic interneurons that inhibit dopaminergic neurons [10]. Desensitization of these nAChRs by chronic nicotine exposure will contribute to elevated dopamine levels in the nucleus accumbens, representing an important functional link to nicotine addiction [11]. Homomeric neuronal nAChRs contain mainly the α7–α10 subunits, are sensitive to the snake venom α-bungarotoxin, and the most abundant and important of these receptors is the α7 nAChR [12]. Levels of this receptor are high in the hippocampus, where it can fulfill several roles: presynaptic α7 nAChRs on pyramidal cells can augment release of glutamate, owing to the high calcium permeability of this receptor type [13]. In hippocampal GABAergic interneurons, postsynaptic α7 nAChRs regulate inhibition throughout the hippocampus and this has been implied in the processes of short- and long-term potentiation and sensory gating [14, 15]. Accordingly, α7 nAChRs are linked to learning and memory tasks, but also to Alzheimer's disease and schizophrenia [6, 15–17].

The broad spectrum of functions and locations of nAChRs at pre- and postsynapses throughout the nervous system raises the issue of how the activity and subcellular distribution of these receptors are regulated. This review focuses on the roles of tyrosine kinases and tyrosine phosphorylation of nAChRs in distribution (clustering) and activity of nAChRs in the PNS and CNS. We place these phosphorylation mechanisms into the context of scaffolding and cytoskeletal pathways where appropriate and known. Interestingly, tyrosine kinases, even those of the same family, act differently in different tissues. While they regulate the postsynaptic clustering of muscle nAChR at the NMJ, tyrosine kinases increase heteromeric neuronal nAChR activity in adrenal chromaffin cells but diminish α7 nAChR activity in the brain.

The neuromuscular junction: tyrosine kinases of several families cause clustering and stabilization of postsynaptic nAChRs

The high density of muscle nAChRs in the postsynaptic membrane is a hallmark of the NMJ and ensures reliable communication from motor neurons to muscle fibers [5]. nAChR clustering is developmentally regulated and can occur at early stages of NMJ formation, even in the absence of the nerve [18, 19]. Neural input causes a consolidation and formation of new nAChR clusters underneath the nerve terminal, to achieve the typical spatial alignment of pre- and postsynapse [20]. The input of the nerve has two aspects. A positive signal – neurally released agrin – protects and induces new nAChR clusters, while a negative signal – acetylcholine evoking electrical muscle activity – acts to dissolve receptor aggregates that are not protected by local agrin deposition in the synaptic basal lamina [21–23]. It is thought that the combination of these two signals shapes the synapse by aligning nerve terminals with postsynaptic zones in the middle area of muscle fibers [20].

Much evidence has illustrated the crucial role of neural agrin and its downstream signaling pathways in NMJ formation (Fig. 1). Mice lacking neural agrin, although transiently displaying ample nAChR clusters in development, lack nerve-associated clusters and functional NMJs at birth and die from respiratory failure [18, 24]. The receptor for agrin, although still elusive in its complete functional form, contains a receptor tyrosine kinase, MuSK (muscle-specific kinase), as a key component [25, 26]. Agrin triggers kinase activation of MuSK. In the absence of MuSK, nAChR clusters never form, NMJs are dysfunctional and the mice die at birth [18, 25]. Agrin/MuSK signaling not only redistributes existing nAChRs into aggregates but also directs synaptic transcription of the receptor genes [27]. Long thought to depend on neuregulin, nAChR transcription occurs efficiently in mice lacking functional ErbB receptors for neuregulin in muscle [28]. The downstream pathway of agrin/MuSK signaling leading to nAChR clustering features rapsyn, an nAChR-associated scaffolding and anchoring protein, as a central element [29]. Again, the absence of rapsyn leads to a complete absence of nAChR clusters throughout development, although some other aspects of NMJ formation are better preserved in the knockout mice [18, 29]. Interestingly, studies from cultured muscle cells and nAChR-mutant zebrafish revealed that rapsyn does not form synaptic clusters in the absence of nAChRs, showing that rapsyn and associated nAChRs are interdependent players in cluster formation [30, 31].

The agrin-MuSK-rapsyn-nAChR cascade is at the core of postsynaptic NMJ formation and under regulation by a variety of additional players. Recently reviewed by others in detail [32], these include the MuSK-interaction partners dishevelled-1 [33], geranylgeranyltransferase [34] and 14-3-3γ [35], the serine/threonine kinase PAK1 [33], the actin-modulating GTPases Rac, Rho and Cdc42 [36, 37], the MAP-kinase family member JNK and its activating kinase MKK7 [27], and the nAChR- and actin-binding protein adenomatous polyposis coli (APC) [38]. This protein array highlights the reorganization of actin and the activation of MAP kinase-family members as key aspects in synaptic pathways, triggered by agrin/MuSK, that lead to nAChR clustering and transcription.

Another important aspect in regulation of the agrin/MuSK pathway are downstream nonreceptor tyrosine kinases that interact with MuSK and/or the nAChR (Fig. 1). Strong tyrosine phosphorylation of the nAChR β and δ subunits is triggered by agrin [39–41], and β phosphorylation is required for efficient receptor clustering and cytoskeletal linkage [42]. As it is correlated with increased nAChR-rapsyn binding [43], agrin-induced nAChR phosphory-

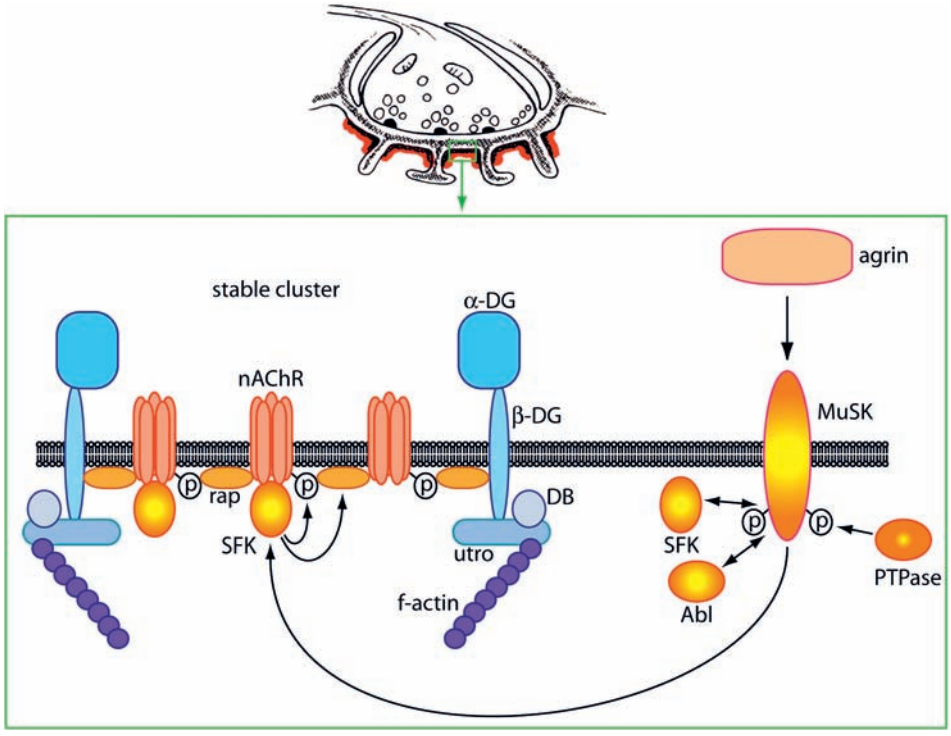

Figure 1. Tyrosine kinase pathways cause phosphorylation and stable clustering of muscle nAChRs in the postsynaptic membrane of the NMJ. Agrin triggers activation of MuSK, SFKs and Abl kinases. These kinases form a complex and engage in mutual phosphorylation; MuSK phosphorylation is also controlled by phosphatases. SFKs associate with the nAChR and maintain its phosphorylation status, nAChR-rapsyn interaction and nAChR-cytoskeletal linkage. Thereby SFKs stabilize clusters of postsynaptic proteins and the postsynaptic apparatus. For more details, see text. MuSK, muscle-specific kinase; SFK, Src-family kinase; Abl, Abelson tyrosine kinase; PTPase, tyrosine phosphatase; rap, rapsyn; p, tyrosine phosphorylation (of nAChR or MuSK); utro, utrophin; DG, dystroglycan; DB, dystrobrevin.

lation may create docking sites – directly or indirectly – for rapsyn to bind the receptor. MuSK does not directly phosphorylate the nAChR but acts through downstream kinases [44]. Surprisingly, a short pulse of agrin followed by efficient wash-off triggers normal nAChR clustering and MuSK activation in cultured myotubes, suggesting that balanced activity of tyrosine kinases and phosphatases that interact with MuSK and/or the nAChR keeps the downstream clustering pathway activated in an autonomous fashion [45]. Abl kinases (Abelson tyrosine kinases 1 and 2), known modulators of actin organization, are localized to the postsynaptic membrane of the NMJ, are interaction partners for MuSK, effect reciprocal tyrosine phosphorylation with MuSK and form a complex with MuSK upon agrin engagement [46]. Abl kinase activity is required for formation of agrin-induced nAChR clusters [46], and can cause phosphorylation of AChR β subunits [45]. Similarly, the Src-family kinase members (SFKs) Src and Fyn interact with and phosphorylate MuSK [47]. SFKs are activated by agrin [39] and cause AChR β phosphorylation triggered by agrin, although with different kinetics than Abl kinases [45]. Src and Fyn, but not the related family member Yes, bind to the nAChR in muscle [48]; in Torpedo electric organ, Src and another SFK-member, the kinase Fyk, bind the nAChR and phosphorylate the receptor [49, 50]. Cluster formation of nAChRs can occur in the absence of SFK function, showing that SFKs are not an essential component in the pathways that cause NMJ formation. Thus, in mice lacking Src and Fyn, NMJs are normal around birth, and agrin induces normal nAChR clustering and nAChR β phosphorylation in cultured *src*$^{-/-}$;*fyn*$^{-/-}$ myotubes [51]. Phosphorylation of MuSK and the nAChR are also regulated by tyrosine phosphatases, as shown by the inhibitor pervanadate, which induces strong phosphorylation of these two proteins in muscle cell cultures [47, 52]. While the phosphatase acting on the nAChR is unknown in muscle, SHP-2 is a good candidate to dephosphorylate MuSK [53].

While the mechanisms described above operate in the initial formation of the NMJ, much less is known about the pathways that stabilize nAChR clusters postnatally and cause mature NMJs to adopt their complex pretzel-shape with nAChRs concentrated at the crests of postjunctional folds. In the postnatal phase of NMJ development, all but one innervating axons are eliminated in a muscle fiber in the process of synaptic elimination. nAChRs are metabolically stabilized and their half-life time increases massively, showing that receptor turnover is altered [54]. nAChR activity is one parameter that determines recep-

tor turnover since inactivated nAChRs are replaced more rapidly [55]. Links to postsynaptic scaffolds are also important as, in the absence of α-dystrobrevin, nAChRs turn over more rapidly [56]. This enhanced degradation may contribute to the postnatal disassembly of NMJs in *α-dystrobrevin*$^{-/-}$ mice [57]. Recycling of internalized nAChRs as such appears to contribute to maintenance of adult NMJs [58]. Taken together, these data suggest that the dynamics of nAChRs at the NMJ as reflected by turnover and recycling are one aspect of postsynaptic stabilization.

Another key aspect lies in the signaling pathways that maintain the adult postsynapse at the NMJ and link it to the underlying cytoskeleton. MuSK is again required [59, 60] and antibodies against MuSK occur in patients with myasthenia gravis [61], implying that MuSK function keeps NMJs intact. However, some of the pathways for NMJ and nAChR cluster maintenance and maturation may not be essential in initial NMJ formation. In mice lacking utrophin, dystrophin, α-dystrobrevin or dystroglycan (components of the postsynaptic utrophin-complex), NMJs form but fail to mature properly [57, 62, 63]. In the absence of α-dystrobrevin, nAChR clusters are normal at birth but increasingly break up postnatally. Similarly, agrin induces normal nAChR clusters in cultured *α-dystrobrevin*$^{-/-}$ myotubes, but the clusters disperse rapidly when agrin is removed from the culture medium [57]. These data highlight the utrophin-complex as mediator of synaptic stability. Additional key players are SFKs. Following agrin or laminin withdrawal from *src*$^{-/-}$*;fyn*$^{-/-}$ myotubes, the induced nAChR clusters rapidly disassemble [51, 64], and β nAChR phosphorylation as well as rapsyn-nAChR interaction decrease in parallel [65]. Under the same experimental circumstances, co-extensive clusters of other postsynaptic proteins are lost together with nAChR clusters, showing that SFKs hold proteins of the postsynaptic apparatus together in aggregates [65]. In addition, SFKs regulate the overall amount of rapsyn protein and the cytoskeletal link of the nAChR. Turnover of nAChRs, however, is not affected in *src*$^{-/-}$*;fyn*$^{-/-}$ myotubes, indicating that mechanisms independent of nAChR degradation or insertion conduct postsynaptic stability [117]. *In vivo*, expression of dominant-negative or overactive Src constructs in adult mouse muscle causes disassembly of postsynaptic nAChR pretzels, in parallel with dislocation of synaptic nuclei and changes in subsynaptic α-tubulin distribution [65]. These studies show that SFKs are essential to keep the postsynaptic apparatus of the NMJ intact, by maintaining nAChR phosphorylation, cytoskeletal interaction and binding to rapsyn (Fig. 1).

Taken together, these results about tyrosine kinase function at the NMJ show that MuSK and Abl are key kinases in the formation of the NMJ and nAChR clusters, while SFKs are important in maintenance of these structures. Regulation of the cytoskeleton, particularly actin polymerization, is likely to be a common link between the two aspects.

Modification of the nAChR activity and ACh-evoked currents by tyrosine phosphorylation – a mechanism that is prominent for many other ionotropic neurotransmitter receptors such as those for glutamate [66] or GABA [67] – plays a minor role, if any, at the NMJ. Strong tyrosine phosphorylation of nAChRs in Torpedo electric organ membranes *in vitro* produces small changes in the receptor desensitization kinetics [68], an effect produced also by phosphorylation of nAChR serine or threonine residues [69]. The relevance of this modification for nAChR function *in situ* at NMJs remains unclear.

The PNS: SFKs activate heteromeric neuronal nAChRs and catecholamine secretion in adrenal chromaffin cells

Neuronal nAChRs have prominent postsynaptic functions in the PNS. Like neuromuscular nAChRs, they often are clustered in the postsynaptic membrane of cholinergic synapses and mediate fast synaptic transmission [4]. Examples are the ciliary and superior cervical ganglia, where the nAChRs functionally connect pre- and postganglionic neurons via cholinergic excitation. In chick ciliary neurons, clustering of heteromeric nAChRs (receptors containing the α3, α5, β2 and β4 subunits) in the postsynaptic density depends on signals within the cytoplasmic loop of α3 subunits [70] and on postsynaptic functioning of APC protein [71]. One APC-interaction partner in this process is PSD-93, a member of the PSD-95-family of PDZ-domain-containing scaffolding proteins [71]. PSD-93 and PSD-95 associate with the nAChRs, and interfering with their function reduces spontaneous EPSC frequency and nicotine-induced long-term phosphorylation of the transcription factor CREB in ciliary neurons, showing that PSD-93 and PSD-95 form a scaffold operative in nicotinic signaling [72]. This is an interesting parallel to excitatory synapses in the brain, where PSD-95-family members provide prominent postsynaptic functions [73]. Another common player is APC protein, which is essential for clustering of both neuronal nAChRs at the chick ciliary ganglion [71] and muscle nAChRs at the NMJ [38], most likely by modulating cytoskeletal dynamics.

The chick ciliary ganglion also contains homomeric neuronal nAChRs, particularly α7 receptors [12]. Although excluded from the actual postsynaptic density and located more perisynaptically, α7 nAChRs mediate considerable postsynaptic currents in ciliary neurons [12]. Spine-like appendages exist on the soma of ciliary neurons, are folded into mats and contain high α7 concentrations in clusters [74]. Actin filaments colocalize with the α7

nAChRs [75], and retention of somatic spines and stable amounts of α7 nAChRs are dependent on the integrity of the actin cytoskeleton [76]. Actin dispersal accelerates rundown of α7 nAChR function, whereas actin stabilization diminishes the rundown [76, 77]. Besides actin, another mechanism involved in clustering of α7 nAChRs in ciliary ganglion neurons is represented by lipid rafts. α7 receptors colocalize well with ganglioside GM1, a marker lipid of the rafts and binding partner for cholera toxin, and biochemical raft preparations contain high amounts of α7 nAChR [75]. Extraction of cholesterol, leading to lipid raft dispersal, caused a fragmentation of α7 nAChR clusters into small microaggregates on the surface of ciliary neurons [75]. In summary, actin filaments and lipid rafts are part of the machinery that keeps α7 nAChRs in clusters at their perisynaptic location.

In rodent superior cervical ganglion neurons, cholinergic interneuronal synapse formation and synaptic clustering of neuronal nAChRs require agrin [78]. nAChR clustering is independent of rapsyn, which is expressed only a low levels [79], although transfection of rapsyn increases the stability of transfected nAChR subunits at the surface of heterologous cells [80]. In mice lacking PSD-93, cholinergic synapses form properly in the superior cervical ganglion, but nAChR clusters disassemble much faster after denervation [81], suggesting that PSD-93 regulates synaptic stability. Taken together, these recent data from ciliary and superior cervical ganglia show that both similarities and differences exist in postsynaptic formation and stability between the cholinergic NMJ, cholinergic autonomic ganglia and CNS glutamatergic synapses.

Neuronal heteromeric nAChRs are also found in neuroendocrine target organs like the adrenal gland, where receptors of the $\alpha3\alpha5\beta4$ type reside on the surface of chromaffin cells and receive input from the innervating cholinergic neurons of the sympathetic nervous system [9]. Binding of either acetylcholine or nicotine to these nAChRs opens the receptor channel, leading to cation influx. The resulting depolarization of the chromaffin cellular membrane opens L-type voltage-gated calcium channels, causing calcium entry that triggers the release of catecholamines via regulated secretory mechanisms into the blood stream (Fig. 2) [82, 83].

Earlier studies had shown that SFKs (Src, Fyn and Yes) are highly expressed in chromaffin cells of the adrenal medulla [84–87], and that serine/threonine as well as tyrosine kinases influence chromaffin cell secretion [88]. Thus, the tyrosine kinase inhibitors genistein and tyrphostin 23 block most catecholamine secretion in chromaffin cells stimulated with nicotine, KCl (to achieve depolarization) or calcium ionophores [88, 89]. In addition, overexpression of Src reversed the inhibitory effect of vaccinia virus on secretion [90]. Since SFKs localize to the cell membrane as well as to secretory vesicles and are known to be able to regulate secretion [91–94], SFKs could, in principle, act at multiple levels in the pathway leading from nAChR activation to hormone release.

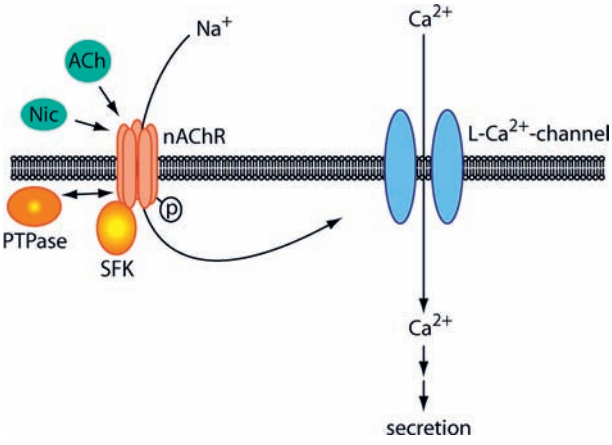

Figure 2. Tyrosine kinases act in ACh- or nicotine-induced catecholamine secretion in adrenal chromaffin cells by activating $\alpha3\alpha5\beta4$ nAChRs. Innervating neurons release ACh, activating $\alpha3\alpha5\beta4$ nAChRs, leading to cation influx, membrane depolarization and opening of L-type voltage-gated calcium channels. Calcium causes catecholamine release via downstream signaling steps and regulated secretion. SFKs associate with nAChRs and activate the receptor, while phosphatases deactivate it. ACh, acetylcholine; Nic, nicotine; SFK, Src-family kinase; PTPase, tyrosine phosphatase; p, tyrosine phosphorylation; L-Ca^{2+}-channel, L-type voltage-gated calcium channel.

Recent data show that a major regulatory step in this pathway acts at the level of the nAChRs (Fig. 2). Pharmacological inhibition of SFKs or expression of a kinase-dead Src construct reduces the peak amplitude of nicotine-induced currents in chromaffin or heterologous cells that express the $\alpha3\alpha5\beta4$ nAChR [95]. Conversely, inhibition of tyrosine phosphatases by pervanadate, or expression of catalytically activated mutant Src yields enhanced current amplitudes. Src and Fyn (but not Yes) co-precipitate with the $\alpha3\alpha5\beta4$ nAChR and the receptor is tyrosine-phosphorylated [95] – all very similar to the situation with the muscle nAChR, which is also phosphorylated on tyrosine residues and associates with Src and Fyn but not Yes [48]. While it remains unclear whether SFKs directly phosphorylate the chromaffin $\alpha3\alpha5\beta4$ nAChR, these recent data show that a balance of SFKs and phosphatases, whose identity is unknown, regulates the activity of these nAChRs by opposing actions. SFKs act to activate the receptor while tyrosine phosphatases deactive it. This model is supported by biochemical data showing the presence of SFKs, phosphatase activity and $\alpha3\alpha5\beta4$ nAChR in large multimeric complexes [96]. The role of the phosphorylation of the nAChR itself in this regulatory process, the signals that regulate SFK and/or phosphatase activity, and the significance of nAChR phosphorylation and SFKs in targeting steps within the chromaffin cells all remain unknown.

The CNS: tyrosine phosphorylation and SFKs negatively regulate the activity of homomeric nAChRs in the brain

Mechanisms of intracellular targeting and synaptic clustering of neuronal nAChRs are hardly understood in the brain. In fact, not even the distribution of these receptors at and around synapses is completely revealed [4]. Expression profiles of the different nAChR subunits in the brain were recently reviewed by others [97]. Here we focus on the two nAChRs subtypes that are most abundant and important in the brain, the heteromeric $\alpha4\beta2$ nAChR and the homomeric $\alpha7$ nAChR. The $\alpha4\beta2$ receptor is widespread throughout the nervous system, with its highest levels in the thalamus, substantia nigra pars compacta (SNc), ventral tegmental area (VTA) and other brain regions. The $\alpha7$ nAChR was found highly concentrated, amongst other areas, in the hippocampus, hypothalamus, olfactory bulb and the amygdala [98]. In studies on the rat SNc and VTA, the $\alpha4$ subunit was detected in a majority of the dopaminergic neurons [99, 100] and also in some GABAergic cells [101]. On an ultrastructural level the locus could be pinpointed to the postsynaptic density of the dendrites of SNc neurons. In addition to this postsynaptic localization, there are also hints to extra- or perisynaptic occurrence of the $\alpha4\beta2$ receptor [99, 100]. The ultrastructural distribution of the $\alpha7$ nAChR in the brain seems to be quite diverse. Besides presynaptic detection, there is ultrastructural evidence for post- and perisynaptic localization in hippocampal neurons [102, 103]. By electrophysiological assessments, $\alpha7$ nAChRs were found to mediate prominent cholinergic synaptic input onto GABAergic interneurons in the hippocampus [104, 105], thereby regulating inhibition within the hippocampal network [106]. The postsynaptic localization of nAChR in hippocampal GABAergic interneurons was confirmed in dissociated hippocampal cell cultures, where clusters of $\alpha7$ nAChR were visible [107]. The scaffolding and clustering machinery of neuronal nAChRs in the CNS, at presynaptic or postsynaptic locations, is completely unidentified. Indirect cues known are NMDA receptor activity and neurotrophins such as brain-derived neurotrophic factor (BDNF) or nerve growth factor (NGF), which all act to maintain and trigger clusters of $\alpha7$ nAChRs on cultured hippocampal GABAergic interneurons [107]. These neurotrophins can also induce $\alpha7$-subunit mRNA transcription in PC12 cells [108] and thereby probably represent a mechanism to influence receptor expression rather than clustering.

Tyrosine phosphorylation in the brain is known to have an impact on neurotransmitter receptor functioning. Phosphorylation of the NMDA receptor is an essential mechanism to influence long-term potentiation (LTP) in the hippocampus, the key process correlated with learning and memory [109]. Src-family kinase members play an important role in NMDA receptor-mediated LTP [109] and spatial memory [110]. Regulation of hippocampal LTP can also be influenced by the $\alpha7$ nAChR. Upon acetylcholine application onto GABAergic interneurons in hippocampal slice cultures and concomitant stimulation of the Schaffer collaterals, long-term depression was evoked in CA1 pyramidal neurons [14]. On the other hand application of acetylcholine to the pyramidal cells, together with Schaffer collateral stimulation, facilitated LTP induction in the pyramidal cells [14]. This provokes the question if there is any connection between SFK members and the $\alpha7$ nAChR. A link between $\alpha7$ nAChRs and tyrosine kinases (ErbB family) could be shown in hippocampal neurons where activation by neuregulin led to an increase in α-bungarotoxin binding sites and higher currents mediated by $\alpha7$ nAChRs [111]. This long-term effect, however, seems to be rather an indirect effect on the receptor by enhancing $\alpha7$ expression; it cannot underlie the rapid and precise timing necessary for the $\alpha7$-mediated effects on LTP and LTD as described above.

Two recent papers provide new insights into regulatory mechanisms of the $\alpha7$ nAChR by tyrosine phosphorylation and dephosphorylation [112, 113] (Fig. 3). Both show that inhibition of tyrosine kinases (by the broad-spectrum inhibitor genistein) enhances the response of $\alpha7$ nAChRs upon agonist presentation, whereas general inhibition of tyrosine phosphatases (using pervanadate) reduces the activity of the $\alpha7$ receptors – all measured in several model systems such as Xenopus oocytes, SH-SY5Y neuroblastoma cells and hippocampal interneurons from slice cultures [112, 113]. In addition Charpantier and colleagues [112] found that the $\alpha7$ nAChRs interact with and are phosphorylated by members of the Src-family kinases, that only surface (not internal) $\alpha7$ receptor in

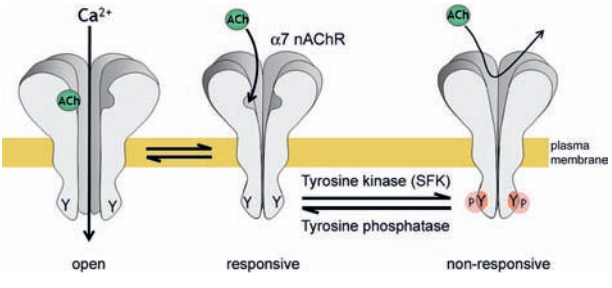

Figure 3. Model of tyrosine kinase and phosphatase pathways that regulate $\alpha7$ nAChR activity. Tyrosine kinases (inhibited by genistein) such as SFKs phosphorylate the $\alpha7$ nAChR and cause a decrease in ACh-evoked current. Unidentified tyrosine phosphatases (inhibited by pervanadate) dephosphorylate the receptor and produce a current enhancement. Thus tyrosine phosphorylation of $\alpha7$ nAChRs negatively regulates ACh-induced current. As the $\alpha7$ receptor is formed by five homologous subunits and each subunit harbors two putative tyrosine phosphorylation sites, the whole receptor contains up to ten sites for tyrosine phosphorylation. For details, see text. ACh, acetylcholine; SFK, Src-family kinase; Y, tyrosine phosphorylation site.

the cell membrane is detected in a tyrosine-phosphorylated state, and that SFK inhibition increases α7 receptor activity. Mutant α7 receptors, lacking cytoplasmic tyrosine phosphorylation sites, showed enhanced acetylcholine-evoked currents, suggesting that the phosphorylation state of the α7 receptors determines the responsiveness towards agonists [112]. Since the agonist sensitivity was not significantly affected in any of the tests, the data suggest that a balance of SFKs and unidentified tyrosine phosphatases determines the overall activity of the α7 receptors, most likely by shifting receptors between responsive and non-responsive states (Fig. 3). Overall, tyrosine phosphorylation and SFKs mediate a negative effect on α7 activity.

One issue is whether the increase in α7 receptor functioning upon dephosphorylation derives from a higher amount of receptor on the surface induced by scaffolding or transport mechanisms or whether surface receptor amounts remain unaltered but receptor function is affected. Even though the α7 nAChR was proposed not to undergo rapid exocytosis [114] and its potentiation upon dephosphorylation is independent of the actin cytoskeleton [113], treatment with botulinum neurotoxin decreased genistein-induced α7 receptor potentiation [113]. Botulinum toxin acts by cleaving SNAP25, a component of the SNARE machinery, and prevents exocytosis [115]. However, as both papers show that only 50% of the functional α7 receptor reside in intracellular compartments, the strong increase in α7 nAChR activity induced by genistein (up to sixfold higher current amplitudes) cannot solely be explained by receptor trafficking to the cell surface [112, 113]. Furthermore, genistein, SFK inhibitors and mutation of tyrosine phosphorylation sites did not affect the clustered distribution of α7 nAChR at the surface of SH-SY5Y cells or dissociated hippocampal neurons [112] (A. Wiesner and C. Fuhrer, unpublished observations).

In summary, tyrosine phosphorylation of α7 nAChRs provides a mechanism to regulate receptor activity, in that phosphorylation and SFKs negatively affect the receptor, while dephosphorylation causes a potentiation. While a minor part of this effect may be due to α7 nAChR trafficking [113], the main regulation seems to occur independently of receptor targeting or redistribution, possibly by affecting the balance between responsive and non-responsive receptors at the cell surface (Fig. 3).

Concluding remarks and outlook

In contrast to muscle nAChRs at the NMJ, tyrosine phosphorylation of neuronal nAChRs by members of the Src-family kinases has not been reported to affect receptor distribution, clustering or stability, but receptor function. Unlike in adrenal chromaffin cells, tyrosine phosphorylation by SFKs has a negative regulatory effect for nAChRs in the brain, exemplified by α7 nAChRs. Thus, in both the PNS and CNS, SFKs are positioned to regulate the activity of neuronal nAChRs, although the actual effects are diverse.

At the NMJ, the full spectrum of pathways that stabilize postsynaptic nAChRs by integrating downstream kinases, cytoskeletal mediators and scaffolding proteins, remains to be identified. NMJ and nAChR cluster stabilization are particularly dynamic during the phase of synaptic elimination after birth. Without doubt, future research will identify the underlying molecular players as well as signaling and trafficking pathways. Mechanisms dependent upon tyrosine kinases *versus* phosphatases represent promising candidates.

For neuronal nAChR regulation in the PNS, scaffolds that influence receptor clustering and downstream signaling are emerging. Future directions will be to identify more scaffold components and to examine their interplay with tyrosine kinases in clustering and activation of nAChRs, particularly in adrenal chromaffin cells.

For neuronal nAChRs in the CNS, scaffolds or extracellular signals regulating receptor clustering have yet to be identified. For regulation of nAChR activity, it is important to note that the neuronal network in the CNS is vulnerable to calcium-induced excitotoxicity. Therefore, a precise control of calcium influx into the cell is necessary. Due to the fact that the α7 nAChR is highly permeable to calcium ions, a sensitive and rapidly reacting mechanism to control its activity seems of advantage. This requirement could be fulfilled by tyrosine phosphorylation. As Src-family members are known to be involved in neuroprotection pathways, even those triggered by α7 nAChR [116], SFKs could provide an effective negative feedback loop on α7 nAChRs, to balance neuroprotection *versus* calcium toxicity. This aspect and the signals regulating SFK activity are topics of future research.

Tyrosine phosphorylation also regulates processes of synaptic plasticity in the brain, such as hippocampal LTP. Since both α7 nAChRs and SFKs can regulate aspects of such potentiation, it will be interesting to learn whether tyrosine phosphorylation, α7 receptors and SFKs collaborate somehow in the fine tuning of synapse plasticity in neuronal circuits.

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