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Layer-Dependent Modulation of Mouse Insular Synaptic Activities by Nicotinic Acetylcholine Receptors

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Abstract

It has been evident that the insular cortex (IC) is involved in formation of nicotine addiction. However, its neural mechanisms remain largely unclear. Therefore, we have recently investigated how activation of nicotinic acetylcholine receptors (nAChRs) affects synaptic activities such as synaptic transmission and plasticity in layer 3, 5 and 6 (L3, L5 and L6, respectively) pyramidal cells (PCs) of the IC in mice. Furthermore, we investigated which cell types have functional nicotinic receptors in L3, L5 and L6 of the IC. We have found that activation of nicotinic receptors layer-dependently modulates synaptic transmission and plasticity in L3, L5 and L6 PCs of the mouse IC. In this short review, I describe our experimental evidence which may contribute to neuronal mechanisms for addiction to tobacco smoking.

Keywords

Nicotinic receptor, Synaptic transmission, Synaptic plasticity, GABA interneuron, Pyramidal neuron, Insular cortex

Introduction

The insular cortex (IC) plays a number of roles including processing of visceral and somatosensory inputs, craving, olfaction, language, audition, motivation and emotions [1-3]. It has recently been accepted that the IC is a pivotal brain area, which is responsible for nicotine addiction [4, 5]. In a human study, it has been shown that smokers with ischemic damage to the insula were able to easily stop smoking without relapse or cravings [6]. In previous studies using rats, it has been demonstrated that pharmacological inactivation of the agranular and granular IC and electrical stimulation of the insular region attenuated nicotine self-taking and reinstatement of nicotine seeking [7, 8]. However, synaptic and cellular mechanisms in the IC, which are associated with addiction to nicotine, have not been examined.

Nicotinic receptors are cholinergic receptors that form ligand-gated ion channels. The neuronal subtypes are homomeric or heteromeric combinations of twelve different nicotinic receptor subunits (α2-α10 and β2-β4) [9]. In the cerebral cortex of mice and rats, α4β2 and α7 nicotinic receptors are predominantly present [10]. The cholinergic system in the prefrontal cortex (PFC) plays a role in memory and cognition. Thus, the roles of nicotinic receptors in synaptic activities have been well investigated in the PFC [11-14]. In L3 and L6 of the mouse PFC, excitatory synaptic inputs are not affected by activation of nicotinic receptors [14] whereas those to L5 PCs are increased by activation of β2 nicotinic receptors, presumably via thalamocortical inputs [14, 15]. On the other hand, nicotinic receptor activation enhances GABAergic synaptic transmission in L3

and L5 PCs of the mouse PFC [14, 16] while it has almost no effect on GABAergic synaptic transmission in L6 PCs [14]. In addition, an application of nicotine depresses spike-timing-dependent plasticity (STDP) in L3 and L5 PCs of the mouse PFC, although it facilitates STDP in L6 PCs [12, 14, 16, 17]. Thus, in the mouse PFC, a layer-specific expression of nAChRs would be related with a layer-specific alteration of synaptic activities in PCs [11]. However, there was no information about how activation of nicotinic receptors affects synaptic activities in the IC.

Layer-dependent modulation of synaptic transmission by nicotinic receptors in the mouse IC

It has been demonstrated that a majority of L2/3 PCs in the PFC, visual cortex, somatosensory cortex and motor cortex do not have functional nicotinic receptors [14, 18-20]. In agreement with these findings, most of L3 PCs in the IC (26 of 29 cells, 90%) did not have functional nicotinic receptors (Figure 1) [21]. Indeed, glutamatergic synaptic transmission in L3 PCs of the IC was not affected by nicotinic receptor activation [21]. In L3 of the IC, an about half of fast-spiking interneurons (6 of 11 cells, 55%) expressed α7 nicotinic receptors and most of non-fast-spiking interneurons expressed α7 and/or β2 nicotinic receptors (α7/β2: 11 of 28 cells; 39%, α7: 5 of 28 cells; 18%, β2: 10 of 28 cells; 36%, Figure 1) [21]. These results are comparable to those obtained from L2/3 of the mouse PFC [14, 16]. Consistent with these findings, the enhanced GABAergic synaptic transmission in L3 PCs of the IC caused by nicotinic receptor activation was largely abolished by DHβE (dihydro-β-erythroidine hydrobromide, an antagonist of β2 nicotinic receptors) and was moderately abolished by MLA (methyllycaconitine citrate, an antagonist of α7 nicotinic receptors) [21].

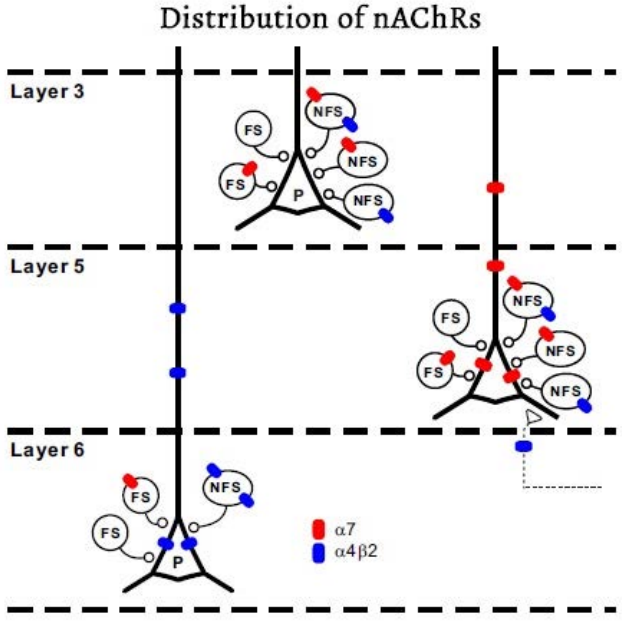

Figure 1: Distribution of nicotinic receptors in L3, L5 and L6 of the mouse IC.

L3 PCs have no functional nicotinic receptors. Nearly half of L5 PCs express functional α7 nicotinic receptors. L6 PCs have functional β2 nicotinic receptors. Glutamatergic inputs to L5 PCs are modulated by β2 nicotinic receptors that are presumably expressed in thalamocortical fibers. Glutamatergic inputs onto L6 PCs are modulated by β2 nicotinic receptors. GABAergic inputs onto L3, L5 and L6 PCs are modulated largely by β2 nicotinic receptors expressed in non-fast-spiking interneurons and to a minor extent by α7 nicotinic receptors expressed in fast-spiking and non-fast-spiking interneurons. P, PCs; FS, fast-spiking interneurons; NFS, non-fast-spiking interneurons.

It has been reported that L5 PCs of the PFC and hippocampal CA1 PCs have functional nicotinic receptors [14, 22], although L5 PCs of the visual cortex, somatosensory cortex and motor cortex do not have functional nicotinic receptors [18-20]. Consistent with the former observation, we found that an about half of L5 PCs (27 of 54 cells, 54%) had α7 nicotinic receptors in the mouse IC (Figure 1) [23]. This observation may indicate that application of nicotine directly enhances excitatory synaptic transmission in L5 PCs via postsynaptic α7 nicotinic receptors. However, our data showed that spontaneous excitatory postsynaptic currents (sEPSCs) in L5 PCs of the mouse IC were not blocked by MLA but were abolished by DHβE, revealing that the excitatory synaptic transmission in L5 PCs of the mouse IC is enhanced by activation of β2 nicotinic receptors but not α7 nicotinic receptors [23]. This observation suggests that α7 nicotinic receptors are not activated by the relatively slow increases in concentrations of ACh (bath application) [14]. The IC receives afferent inputs from nuclei of the thalamus [24], and neuronal nicotinic receptor subunit mRNAs of α4 and β2 are strongly expressed in the thalamic nuclei and thalamocortical fibers [25]. Therefore, it is likely that activation of β2 nicotinic receptors located in thalamocortical axon terminals within the IC increases release of glutamate [15]. In L5 of the mouse PFC, distinct subtypes of interneurons have several types of nicotinic receptors: an about half of fast-spiking interneurons express α7 nicotinic receptors, and a majority of non-fast-spiking interneurons, such as low-threshold spiking neurons and regular-spiking non-pyramidal neurons, express α7 and/or β2 nicotinic receptors [14, 16]. In L5 of the mouse IC, a majority of non-fast-spiking interneurons (22 of 30 cells, 73%) expressed β2 nicotinic receptors while a minority of non-fast-spiking interneurons (1 of 30 cells, 3%) and an about half of fast-spiking interneurons (6 of 13 cells, 46%) expressed α7 nicotinic receptors (Figure 1) [23]. It has been shown that GABAergic synaptic transmission in L5 PCs of the PFC was remarkably augmented by activation of nicotinic receptors [14, 16]. Consistent with these findings, GABAergic synaptic transmission in L5 PCs of the mouse IC was markedly enhanced by activation of nicotinic receptors, predominantly through activation of β2 nicotinic receptors and to a small extent through activation of α7 nicotinic receptors [23]. This enhancing effect of nicotinic receptors on GABAergic synaptic transmission is likely to be mediated by β2 nicotinic receptors in non-fast-spiking interneurons.

Unlike L3 and L5 PCs, a majority of L6 PCs (19 of 21 cells, 90%) in the mouse IC expressed functional β2 nicotinic receptors (Figure 1) [21]. These results are similar to those found in L6 PCs of the PFC [14, 26]. Consistent with the expression of β2 nicotinic receptors, excitatory synaptic transmission in L6 PCs of the IC was abolished by DHβE [21]. It is believed that

L6 PCs in the neocortex receive sparse but potent excitatory inputs from within layer 6 as well as from other cortical layers [27]. Thus, it is indicated that the enhancement of excitatory synaptic inputs to L6 PCs by nicotinic receptor activation is brought about by excitatory inputs from neighboring PCs within L6 as well as those from L5 PCs. In L6 of the mouse IC, an about half of fast-spiking interneurons (4 of 10 cells, 40%) expressed functional α7 nicotinic receptors and a majority of non-fast-spiking interneurons (18 of 21 cells, 86%) expressed β2 nicotinic receptors (Figure 1) [21]. The GABAergic synaptic transmission in L6 PCs of the IC was augmented by activation of β2 nicotinic receptors. These observations suggest that the enhanced GABAergic synaptic transmission is mediated by activation of β2 nicotinic receptors in non-fast-spiking interneurons [21].

Layer-dependent modulation of synaptic potentiation by nicotinic receptors in the mouse insular cortex

In L2/3 and L5 PCs of the mouse PFC, GABAergic synaptic transmission is enhanced and the STDP is reduced by activation of nicotinic receptors on GABAergic interneurons [12, 16, 17]. In human and mouse L2/3 PCs of the PFC, activation of nicotinic receptors on GABAergic interneurons by cholinergic basal forebrain inputs depresses the STDP [12]. In L5 PCs of the PFC, activation of nicotinic receptors on GABAergic interneurons increases inhibitory synaptic transmission and depresses the activity of L5 PCs, thereby suppressing the STDP [16, 17] via reducing dendritic calcium signals [16]. Consistent with these observations, activation of nicotinic receptors suppressed synaptic potentiation that was induced by the paired training (80 stimuli to presynaptic terminals at 2 Hz together with depolarization at +30 mV at postsynaptic cells) in L3 PCs of the mouse IC (Figure 2A) [21]. In several brain regions, it has been demonstrated that GABAergic inhibition is enhanced through activation of nicotinic receptors [18, 28, 29]. In the CA1 region of the hippocampus, activation of GABAergic interneurons by nicotine prevented or diminished synaptic potentiation in PCs [22]. Thus, it is likely that activation of nicotinic receptor-expressing GABAergic interneurons located in L3 suppresses synaptic potentiation in L3 PCs through enhancing its threshold. There is also a possibility that nicotinic receptor-expressing GABAergic interneurons located in other cortical layers project onto L3 PCs [27], and depresses synaptic potentiation in L3 PCs. Moreover, nicotinic receptor-expressing PCs located in L5 may activate GABAergic interneurons located in L3 to suppress synaptic potentiation in L3 PCs. Similar to L3 PCs, activation of nicotinic receptors suppressed synaptic potentiation induced by the paired training in L5 PCs of the mouse IC, through enhancing GABAergic synaptic transmission (Figure 2B) [23]. In L3 and L5 PCs of the IC, GABAergic inhibition would be enhanced through activation of nicotinic receptors, thereby inhibiting synaptic potentiation.

As opposed to L3 and L5 PCs, activation of nicotine augmented synaptic potentiation in L6 PCs of the mouse IC (Figure 2C) [21]. The facilitatory effects of nicotinic receptors on synaptic potentiation were totally blocked by DHβE, suggesting an involvement of β2 nicotinic receptors [21]. This result was consistent with the findings obtained in the mouse and human PFC [12]. In the rat entorhinal cortex, it has been shown that nicotine enhanced synaptic transmission and plasticity in L6 PCs by acting on non-α7 subtype nicotinic receptors, most likely β2 subtype nicotinic receptors [30]. In L6 of the PFC, endogenous ACh enhances synaptic potentiation of glutamatergic synapses by activating heteromeric postsynaptic nicotinic receptors containing β2 and α5 subunits [12]. In the future study, it would be necessary to investigate whether α5 nicotinic receptors are present in L6 PCs of the IC, and if this is the case, how α5 nicotinic receptors modulate synaptic activities in L6 PCs of the IC. Taken together, our data indicate that in distinct layers of the IC, the activity of PCs is oppositely regulated by activation of nicotinic receptors either located on presynaptic GABAergic interneurons located on L3 and L5 or on the dendrites in the L6 PCs.

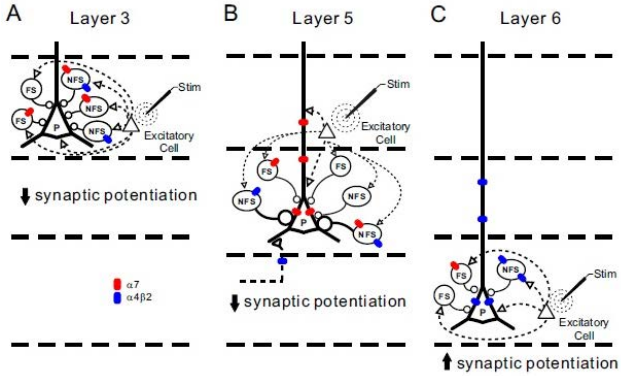

Figure 2: Roles of nicotinic receptors in synaptic potentiation in L3, L5 and L6 of the mouse IC.

(A and B) When LTP-inducing stimuli are applied to L3 in the presence of nicotine, synaptic potentiation in L3 or L5 PCs is suppressed by activation of β2 nicotinic receptors expressed in non-fast-spiking interneurons. (C) When LTP-inducing stimuli are applied to L6 in the presence of nicotine, synaptic potentiation in L6 PCs is enhanced by activation of postsynaptic β2 nicotinic receptors. P, PCs; FS, fast-spiking interneurons; NFS, non-fast-spiking interneurons.

Conclusion

Our data revealed the roles of nicotinic receptors in modulating synaptic activities including synaptic transmission and potentiation in L3, L5 and L6 PCs of the IC in mice [21, 23]. However, it remains unclear how these layer-dependent modulation of synaptic activities by nicotinic receptors is involved in nicotine addiction. It is likely that nicotine modulates local neural circuits within the IC, thereby affecting various cognitive and sensory functions including interoceptive awareness, taste perception/memory, attention and pain processing [1-3]. Future studies regarding the layer-dependent modulation of synaptic activities by nicotinic receptors and its behavioral relationship would be important to understand the neuronal mechanisms for nicotine addiction.

Conflict of Interest

The author declares no conflict of interests.

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