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ORIGINAL ARTICLE

CNS Localization of Neuronal Nicotinic Receptors

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Introduction

Nicotinic acetylcholine receptors (nAChRs) are members of the Cys-loop superfamily of pentameric ligand-gated ion channels, which include GABA (A and C), serotonin, and glycine receptors. Currently, 12 neuronal nAChR subunits have been identified (α2–10 and β2–4) and are generally grouped into α subunits, which contain two adjacent cysteine residues essential for ACh binding, and β subunits, which lack these residues. The majority of neuronal nAChRs fall into two categories: those that bind agonist with high affinity (n*M* concentrations); and those that bind with lower affinity (μ*M* concentrations). The low-affinity receptors are presumably homomeric α7 receptors that are α-bungarotoxin sensitive, whereas α4β2 nAChRs account for >90% of the high-affinity nicotinic receptors in the brain (Whiting and Lindstrom, 1986). Their physiological contributions to neurotransmission, signaling, and behavior are not completely understood. Precise mapping of subcellular and neuroanatomical localizations of neuronal nAChR subunits will help elucidate the physiological role of neuronal nAChRs and their role in nicotine addiction.

Cholinergic neurons are limited to a number of discrete CNS localizations (Woolf, 1991), which make sparse projections to a wide distribution of high-affinity nicotinic receptors in the brain. The behavioral basis for nicotine addiction is complex and varied among tobacco users but includes pleasure, improvement in cognitive function, attention, learning and memory, reduction in stress and anxiety, decreased appetite, relieved depression, and succumbing to social pressures (Picciotto, 2003). Detailed mapping of the anatomical distribution of the primary target of nicotine, the nAChR, would provide clues as to how nicotine impacts on specific behaviors. Much work on addiction has focused on the mesolimbic circuit in the brain, and both α4 and β2 subunits have been implicated in mediating nicotine addiction (Picciotto et al., 1998; Tapper et al., 2004).

A variety of techniques, including antibody labeling, *in situ* hybridization with cRNA probes, and radioactively labeled ligands, have been used to ascertain nAChR distribution in the brain. This paper will review what is known about localization of neuronal nAChRs in the CNS, with an emphasis on α4β2 receptors and their location in the circuits of addiction.

Subcellular Neuronal nAChR Localization

Although most nicotinic receptor signaling appears to be presynaptic in the CNS (McGehee et al., 1995; Gray et al., 1996), there is some evidence for postsynaptic nicotinic signaling (Roerig et al., 1997; Alkondon et al., 1998). The effect of neuronal nAChR activation depends on the subcellular localization of the receptor. Nicotinic receptors expressed on dendrites and soma might mediate fast synaptic transmission and contribute to neuronal excitability through generation of excitatory postsynaptic potentials. (Recent evidence shows cholinergic terminals located remotely from α-bungarotoxin labeling of α7 nAChRs in both ventral tegmental area (VTA) and nucleus accumbens. This suggests that neuronal nAChRs may be activated by paracrine or volume transmission of ACh [Jones and Wonnacott, 2004]). In addition, nAChRs are expressed at axon terminals (Jones et al., 2001), where activation modulates neurotransmitter release through calcium influx and/or terminal depolarization. Nicotinic receptors modulate the release of norepinephrine,

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glutamate, GABA, and dopamine. Currently, the predominant role of nAChRs is thought to be in modulating neurotransmission presynaptically.

CNS Localization of α4β2 Nicotinic Receptors

Radioligand binding, using L-[^{3}H]nicotine, in mice shows moderate binding in many areas of the brain, including the parietal cortex, cingulate cortex, subiculum, substantia nigra, superior colliculus, medial geniculate nucleus, optic nerve dorsal raphe, laterodorsal tegmental nucleus, and retrospenial cortex. Low levels of nicotine binding include the medial septum, nucleus accumbens, caudate putamen, hippocampus, and olfactory tubercle (Marks et al., 1992). The highest density of nicotine binding is in the interpeduncular nucleus, followed by the medial habenula, many thalamic nuclei, ventral lateral and dorsal lateral geniculate nuclei, and optic tract nucleus. Virtually no binding was found in the cerebellum.

There appears to be very similar, though not exact, correspondence between the distribution of L-[^{3}H]nicotine binding and α4 mRNA expression in the CNS (Wada et al., 1989; Marks et al., 1992). The discrepancy might be due to targeting of α4 subunit proteins to axons and presynaptic terminals while the mRNA resides in the soma. Immunolabeling of β2 nAChR subunits with MAb270 also shows an expression pattern very similar to L-[^{3}H]nicotine binding (Clarke et al., 1985; Swanson et al., 1987). Immunolabeling with MAb270 shows discrete expression at all levels of the brain and spinal cord, with particularly strong labeling in the interpeduncular nucleus, the medial habenula, the thalamus, and the superior colliculus.

α4 and β2 nAChR subunit mRNAs are detected in the nervous system as early as E11 in rats. Both subunit mRNAs are expressed in the CNS and the PNS. However, α4 expression is down-regulated in the PNS to undetectable levels by E15, whereas β2 mRNA expression is maintained in the PNS until adulthood (Zoli et al., 1995).

Localization of α4β2 nAChRs in the Mesolimbic System

Although nAChRs are expressed in many CNS regions, nicotine's major addictive effects are thought to be mediated through the dopaminergic mesocorticolimbic pathways. Dopaminergic neurons, originating in the VTA, project to nucleus accumbens and prefrontal cortex. Three cell types in the VTA, dopaminergic neurons, GABAergic neurons, and glutamatergic axonal terminals from the prefrontal cortex, all contain nicotinic receptors (Mansvelder and McGehee, 2000; Mansvelder et al., 2002). The balance of excitatory and inhibitory inputs onto VTA dopaminergic neurons affects dopamine release in the nucleus accumbens and is likely critical for the reinforcing effects of nicotine. α4 and β2 mRNAs are expressed in nearly every dopaminergic and GABAergic neuron in the VTA, whereas α2 is not expressed at all (Klink et al., 2001). α7 nAChR subunit mRNA is found in 40% of either cell type; β4 is sparsely distributed in both GABAergic (25%) and dopaminergic neurons (12%). Meanwhile, GABAergic neurons express mRNA from a similar variety of neuronal nAChRs as dopaminergic neurons. However, α5, α6, and β3 mRNAs express at lower percentages of GABAergic (<25%) than dopaminergic (>70%) neurons.

The VTA and substantia nigra pars compacta (SNpc) contain moderate to high levels of α4β2 nAChR mRNA (Wada et al., 1989). Antibody labeling of α4 and β2 subunits in the soma and dendrites of the SNpc shows colocalization in >90% of tyrosine hydroxylase–positive dopaminergic neurons (Arroyo-Jimenez et al., 1999; Jones et al., 2001). Immunoelectron microscopic studies show that α4 subunits are located in the perikarya and dendritic shafts but not spines, and very scarcely but authentically in postsynaptic membranes in dopaminergic neurons (Arroyo-Jimenez et al., 1999). β2 subunits show a similar localization pattern in dopaminergic neurons in the SNpc but also show immunoelectron microscopic evidence of presynaptic localization in dopaminergic axonal terminals in the dorsal striatum (Jones et al., 2001). Immunoprecipitation and 6-hydroxydopamine lesioning experiments suggest that α4 subunits are also located in dopaminergic projections in the dorsal striatum (Zoli et al., 2002). However, given the apparent similarity of ion channel composition and projections of dopaminergic neurons in the VTA and SNpc, it is presently unknown why nicotine preferentially stimulates dopamine release from the mesoaccumbens but not the nigrostriatal system (Imperato et al., 1986; Benwell and Balfour, 1997). An advancement of techniques, using a combination of modern mouse genetic engineering, genetically encoded fluorescent proteins as tags, and a modern laser scanning confocal or two-photon microscope, can shed new light on this unresolved issue.

Conclusions

In addition to knowledge on nAChR localization gained using conventional techniques of ligand binding, antibody labeling, and *in situ* hybridization, the field would greatly benefit from the exploitation of

modern imaging technology, the use of genetically encoded fluorescent proteins, and mutant mice genetic engineering. Knock-in mice with green fluorescent proteins tagged to the GAT1 protein, for example, has enabled high resolution and accurate quantitative imaging in subcellular neuroanatomical regions (Chiu et al., 2002). Improved fluorescence-based imaging microscopy enables deeper tissue penetration, less damage to live tissue with photon excitation, more accurate spectral separation, and high-resolution 3D reconstruction of cells. Such technology would permit imaging of nicotinic receptors in live neurons, deep into brain tissue, and perhaps reveal the spatial arrangement of neuronal circuitry. If using the appropriate pairs of spectral variants of fluorescent proteins tagged to nicotinic receptor subunits, one can also assess receptor assembly at subcellular resolution (Nashmi et al., 2003). These emerging technologies have allowed researchers to examine biology with exquisite detail as never before.

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