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PERSPECTIVE

Getting Past the Asterisk: the Subunit Composition of Presynaptic Nicotinic Receptors That Modulate Striatal Dopamine Release

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Nicotinic acetylcholine receptors (nAChRs), despite wide distribution, do not play a major role in direct mediation of central synaptic transmission. Instead, these receptors are primarily involved in modulating the release of neurotransmitters, including glutamate, GABA, norepinephrine, dopamine, and acetylcholine itself (Role and Berg, 1996; Wonnacott, 1997). Modulation of dopamine release within the striatum is thought to underlie some of the reinforcing and rewarding aspects of nicotine (Dani et al., 2001). Neuronal nAChRs are localized somato-dendritically and on presynaptic terminals of dopaminergic neurons projecting from the substantia nigra (SN) and ventral tegmental area (VTA) to the striatum. Although there is much that we would like to know about these receptors, even the basic goal of understanding subunit composition turns out to be more challenging than it seems. In an article by Salminen et al. (2004) in this issue of *Molecular Pharmacology*, Grady and colleagues combine the use of knockout mice, a selective antagonist, and a dopamine release assay to present the culmination of this effort at the presynaptic terminal. We are now provided with what is likely to be the complete subunit composition of four distinct presynaptic neuronal nAChRs that modulate dopamine release in the striatum.

The nAChRs form as pentameric arrangements of subunits around a central ion channel. Based on protein sequence and gene structure, these subunits are organized into several subfamilies (Corringer et al., 2000). The members of subfamily III (α2-α6, β2-β4) and one member of subfamily II (α7) will concern us here. Members of subfamilies I (α9-α10) and IV (muscle nAChR subunits), and one member of subfamily II (α8), are not expressed in mammalian brain (Corringer et al., 2000; Elgoyhen et al., 2001). Subfamily III is further subdivided into tribes. An agonist-binding site is formed at the interface between a tribe 1 subunit (α2, α3, α4, α6) and a tribe 2 subunit (β2, β4). The receptor is formed from two of these subunit pairs and a fifth subunit from tribe 2 or tribe 3 (α5, β3). Thus, subunit composition determines the pharmacological and functional properties of neuronal nAChRs.

Many subunit combinations have been characterized using exogenous expression systems in the hope that pharmacological and functional properties could be used to identify the subunit composition of receptors in vivo (Role, 1992). Unfortunately, a major problem soon became apparent. For example, if a competitive antagonist is specific for a particular subunit combination in vitro ($\alpha3\beta2$, for example) and is found to antagonize a receptor in vivo, the most that can be said about the in vivo receptor is that it possesses one $\alpha3\beta2$ subunit interface. The other three subunits cannot be definitively identified. This problem led to a provisional nomenclature in which subunits known to be present are stated and unidentified subunits represented by an asterisk (Lukas et al., 1999). The receptor in the example would be designated $\alpha3\beta2^*$, with the asterisk representing three of the five subunits. The goal is to eliminate the asterisk.

Because of the limitations of pharmacological tools then available, presynaptic nAChRs modulating striatal dopamine release initially seemed to be homogeneous (Wonnacott, 1997). This picture began to change with the development of the α-conotoxins (α-Ctx) as tools for investigating neuronal nAChRs (McIntosh et al., 1999). In particular, α-CtxMII was found to be highly selective for the $\alpha3\beta2$ subunit combination (Cartier et al., 1996) and became a useful tool for investigating receptor structure (Harvey et al., 1997). Use of α-CtxMII allowed presynaptic nAChRs on striatal dopaminergic terminals to be divided into two classes: "α-CtxMII–sensitive" and "α-CtxMII–resistant" (Kulak et al., 1997; Kaiser et al., 1998). Because α-CtxMII would be expected to block any nAChR containing the $\alpha3\beta2$ subunit interface, the α-CtxMII–sensitive receptors were identified as $\alpha3\beta2^*$, an exciting advance for the field. Thus it became confusing when deletion of the

ABBREVIATIONS: nAChR, nicotinic acetylcholine receptor; SN, substantia nigra; VTA, ventral tegmental area; Ctx, conotoxin.

α3 subunit failed to eliminate ^{125}I-α-CtxMII binding in terminal regions of the VTA/SN dopaminergic projections (Whiteaker et al., 2002).

This brings us to the story of the "orphans". After the initial cloning of the neuronal nAChR subunit family, progress was made studying many of the subunits. However, a few subunits came to be known as "orphans" because of a lack of demonstrable function. These subunits, α6 (Lamar et al., 1990) and β3 (Deneris et al., 1989), languished as the field moved on. In 1996, the interesting colocalization of α6 and β3 with the β2 subunit in catecholaminergic nuclei was noted, and a role for an α6β2β3 receptor in modulating catecholamine release was proposed (Le Novere et al., 1996). At the time, this proposal was speculative, in that there was no evidence that either α6 or β3 could form functional nAChRs. However, evidence for function was soon obtained (Gerzanich et al., 1997; Groot-Kormelink et al., 1998; Kuryatov et al., 2000). It is worth noting that α6-containing receptors were found to be sensitive to α-CtxMII (Kuryatov et al., 2000). This is not surprising, because α6 is closely related to α3, and residues in α3 found to be critical for α-CtxMII sensitivity (Harvey et al., 1997) are conserved in α6. Other "α3β2-specific" antagonists, such as neuronal bungarotoxin and α-CtxPnIa, have also turned out to block α6-containing receptors (Everhart et al., 2003). The sensitivity of α6-containing receptors to α-CtxMII and the colocalization of α6 and β3 with β2 in VTA dopamine neurons suggested that the α-CtxMII sensitive component of nicotine mediated dopamine release might contain these subunits. Indeed, striatal ^{125}I-α-CtxMII binding is eliminated upon deletion of either α6 or β3 (Champtiaux et al., 2003; Cui et al., 2003)

The array of subunit mRNAs expressed by SN/VTA neurons has been shown to be α3, α4, α5, α6, α7, β2, β3, and β4 (Klink et al., 2001; Azam et al., 2002). This illustrates the complexity of the problem; any or all of these subunits could be involved in forming receptors at presynaptic terminals. A combination of immunoprecipitation and chemical lesioning has identified an α-CtxMII binding class of nAChRs as α6β2(β3) and α4α6β2(β3) and an α-CtxMII-insensitive class as α4β2 and α4α5β2 (Zoli et al., 2002). This work places plausible subunit combinations at the right location but does not address the issue of whether these receptors are functionally involved in mediating dopamine release.

Although mRNA expression and immunoprecipitation studies provide critical information, the combination of receptor subunit knockout mice with pharmacological tools and the dopamine release assay has now allowed definitive conclusions to be reached. The first subunit to be examined using this type of approach was β2. Deletion of the β2 subunit completely eliminated the ability of nicotine to stimulate dopamine release in the striatum when measured using in vivo microdialysis (Picciotto et al., 1998) and in a synaptosomal preparation (Grady et al., 2001). This method has been used more recently to demonstrate the importance of α4 to both the α-CtxMII–sensitive and -resistant receptor classes and the importance of α6 and β3 to the α-CtxMII–sensitive class (Champtiaux et al., 2003; Cui et al., 2003). As shown by Salminen at al. (2004), the use of α-CtxMII is particularly helpful. Subdividing the receptors into the two classes has increased the resolving power of the approach, allowing identification of multiple receptors within each class. Using this approach, Salminen et al. (2004) have demonstrated the presence of β2 in all of these receptors, the presence of α4 in all resistant and some sensitive receptors, and the presence of β3 in most or all sensitive but no resistant receptors. They have also demonstrated the presence of α5 in some resistant but no sensitive receptors. In an important step, they have ruled out a role for α7 and β4 in any of these receptors. Salminen et al. (2004) conclude that the α-CtxMII–sensitive receptors are composed of the α6, β2, and β3 subunits with and without the α4 subunit, whereas the resistant receptors are composed of the α4 and β2 subunits with and without the α5 subunit. The correspondence with the earlier immunoprecipitation studies (Zoli et al., 2002) is striking. Although it is not yet possible to determine whether these receptors reside on the same terminals, intriguing observations suggest that this may be the case. In mice lacking β3, a component of α-CtxMII–sensitive receptors, the α-CtxMII–resistant class is increased. In mice lacking α5, a component of α-CtxMII–resistant receptors, the sensitive class is increased. These observations suggest that knockout of one class may free up subunits such as α4 and/or β2 to take part in the formation of the other class.

So, in presynaptic terminals of dopaminergic neurons projecting to the striatum, we can now account for all known neuronal nAChR subunits. Each subunit has been shown to

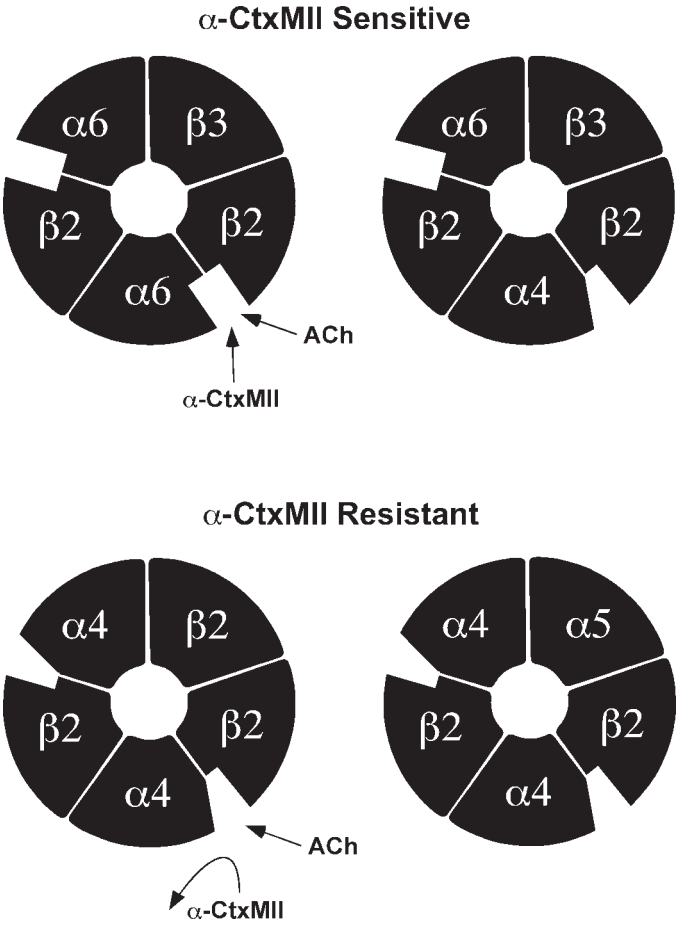

Fig. 1. The subunit composition of striatal presynaptic nicotinic acetylcholine receptors that mediate dopamine release. The α-CtxMII–sensitive receptors have one (α4α6β2β3) or two (α6β2β3) α-CtxMII–sensitive acetylcholine (ACh) binding sites. The α-CtxMII–resistant receptors (α4β2, α4α5β2) each have two α-CtxMII–resistant acetylcholine binding sites.

be either not present in these neurons ($\alpha2$, $\alpha8$, $\alpha9$, $\alpha10$), not significantly involved in forming receptors on the presynaptic terminals ($\alpha3$, $\alpha7$, $\beta4$), or present in at least one of the four identified subtypes ($\alpha4$, $\alpha5$, $\alpha6$, $\beta2$, $\beta3$). Although we cannot rule out minor receptor populations, the studies described by Salminen et al. (2004) enable us to identify the complete subunit composition of four major presynaptic nAChR subtypes in the striatum with reasonable confidence (Fig. 1). The α-CtxMII–sensitive class of receptors consists of $\alpha6\beta2\beta3$ and $\alpha4\alpha6\beta2\beta3$. The α-CtxMII–resistant class consists of $\alpha4\beta2$ and $\alpha4\alpha5\beta2$. Asterisks are no longer required.

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