Neuronal nicotinic receptors: functional correlates of ligand binding sites
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The ligand binding approach to receptor characterization is a convenient way of monitoring receptor numbers and distribution, assessing pharmacological specificity and following the receptor during purification procedures. Ligand binding assays using the high-affinity, pseudo-irreversible antagonist α-bungarotoxin have made an enormous contribution to the understanding of the muscle nicotinic acetylcholine receptor (nAChR) and its counterpart in Torpedo electroplax [1]. The success of α-bungarotoxin in this respect prompted its use to seek out and characterize nAChR in the nervous system. Despite the clearcut identification of specific binding sites for α-bungarotoxin in brain and in autonomic ganglia, this toxin fails to act as a nicotinic antagonist in the majority of instances in which it has been examined [2]. This was surprising since α-bungarotoxin binding sites in nervous tissue are clearly nicotinic in character, binding being displaced by nicotine, acetylcholine and other competitive nicotinic drugs.

In 1980, the first substantial characterization of ligand binding sites for [3H]nicotine in brain membranes was documented [3]. Subsequent studies with [3H]nicotine or other tritiated nicotinic agonists (N-methylcarbamylcholine, or acetylcholine in the presence of atropine) have established the nicotinic character of these binding sites [4]. [3H]Nicotine binding sites and 125I-α-bungarotoxin binding sites do not co-purify [5], they have quite different anatomical distributions in the brain [6] and give distinctive subunit patterns on SDS/PAGE [7]. But given the uncertainty about the receptor status of neuronal α-bungarotoxin sites, it would be imprudent to elevate the tritiated agonist binding sites to nAChR status in the absence of corroborative evidence. Thus it was deemed imperative to complement ligand binding assays with measures of nicotinic function in the brain. This objective is not easily realized. The ligand-gated ion channel nature of nAChR requires intact neurons to demonstrate function, since this depends on ion flux across the plasma membrane. The low density of nicotinic binding sites in the brain (50–100 fmol/mg of protein for both [3H]nicotine- and 125I-α-bungarotoxin-labelled sites) results in weak nicotinic signals. And the sparse distribution of nAChR channels on cell soma has discouraged use of the patch clamp technique [8]. Conversely, the occurrence of presynaptic nAChR on nerve terminals has facilitated the development of a functional, biochemical assay for this class of nAChR in the brain [9]. Thus nicotinic agonists will stimulate the release of transmitters from isolated synaptosomes (Fig. 1a) and this can be conveniently monitored in a superfusion system, after radiolabelling the transmitter pool [10].

The pharmacological profile of nicotine-stimulated [3H]dopamine release from striatal synaptosomes [11] can be compared with the pharmacological sensitivities of the ligand binding sites (Table 1). Because the high-affinity binding of tritiated nicotinic agonists to brain tissue reflects the high-affinity, desensitized state of the nAChR [12], agonist binding cannot be correlated with agonist efficacy in functional assays. However, high stereo-

**Fig. 1**

(a) A schematized nerve terminal illustrating the action of presynaptic nAChR on transmitter release,
(b) a model of the nAChR

Abbreviations: DHβE, dihydro-β-erythroidine; MLA, methyl-lycaconitine.

Abbreviation used: nAChR, nicotinic acetylcholine receptor.
selectivity in favour of the naturally occurring, (−)-enantiomer of nicotine is shown by both nicotine-evoked transmitter release and [³H]-nicotine binding, in contrast to [¹²⁵I]-α-bungarotoxin binding [5, 10]. This correlation is supported by the relative sensitivities to the competitive antagonists neosuqatoxin [11, 13], α-bungarotoxin and methyl-lycaconitine [14, 15]. Clearly the site identified by α-bungarotoxin is not a candidate for the presynaptic nAChR assayed in the perfused synaptosome preparation. However, what started out as a straightforward comparison of two ligand binding sites with nicotinic characteristics is now confounded by the emergent heterogeneity of neuronal nAChR, revealed by molecular cloning [16]. Thus multiple agonist binding (α) and structural (β) subunits have been described in nervous tissue. High-affinity [³H]nicotine binds to the α4 subunit [17]; thus additional probes are necessary to discriminate the other subtypes. Presently, the only pharmacological tool that discriminates between α3 and α4 subunits is neuronal bungarotoxin [18]. This snake toxin is a potent antagonist of α3β2 nAChR complexes expressed in Xenopus oocytes, whereas it inhibits the α4β2 combination only weakly [19]. Therefore we examined neuronal bungarotoxin for its ability to antagonize nicotine-evoked [³H]dopamine release. This toxin has so far given inconsistent results: an initial batch produced 50% inhibition when tested at 100 nM, whereas a second sample was devoid of activity. Similar discrepancies have been reported by other researchers with respect to this toxin [19]. On the other hand, Schulz & Zigmond [20] found a complete blockade of dopamine release from striatal slices by 100 nM neuronal bungarotoxin. A major difference between the latter study and our assay of presynaptic nAChR function is in the concentrations of nicotine used to stimulate the receptors. We employ nicotine concentrations between 1 and 10 µM, approximating to the EC₅₀ of its action [11], whereas Schulz & Zigmond [20] used 100 µM-nicotine. α4β2 nAChR are more sensitive to nicotine than α3β2 nAChR [21]. If both subtypes are present on dopaminergic striatal nerve terminals, only the α4 (neuronal bungarotoxin-insensitive) form may be activated by low concentrations of nicotine.

This dilemma reflects the lack of pharmacological tools for probing nAChR, although this is offset by the fortuitous occurrence of potent and selective natural products [22]. In the absence of subtype-specific ligands necessary for clarification of the relationship between binding sites and functional nAChR, other strategies must be sought. The evidence supports the correlation of [³H]nicotine binding sites with presynaptic nAChR. (i) Experi-
mental and neurological lesions. [³H]Nicotine binding sites in rat striatum are lost as dopaminergic nerves degenerate following 6-hydroxydopamine lesions [23]. This accords with localization of the binding sites on dopaminergic terminals: post-synaptic receptors would be expected to proliferate under these conditions (denervation supersensitivity). Neuronal degeneration that accompanies Parkinson’s and Alzheimer’s diseases is also accompanied by a loss of [³H]nicotine binding sites from the terminal fields affected in these conditions [24].

(ii) Subcellular fractionation. Resolution of plasma membranes from synaptosomes on Percoll gradients results in [³H]nicotine binding sites predominating in the synaptosome fractions [25]. This contrasts with recovery of most muscarinic sites (labelled with [³H]quinuclidinylbenzilate) in the plasma membrane fractions. (iii) Modulation by chronic agonist treatment. Chronic administration of nicotine agonists to rats results in the up-regulation of tritiated agonist binding sites [27]. The specificity of this response was evident from the lack of effect on K⁺-evoked release. This result is therefore consistent with the identity of presynaptic nAChR with [³H]nicotine binding sites. The occurrence of presynaptic nAChR is also supported by immunohistochemical experiments that reveal nAChR labeling in axons [28]. However, the antibody used (mAb 270) recognizes the structural subunit β2, and therefore this study does not distinguish between α3β2 and α4β2 subtypes of nAChR. Similarly, in situ hybridizations recognize the expression of both α3 and β2 transcripts in substantia nigra, for example, and it has been proposed that one subtype may be transported to presynaptic locations [29]. Because mRNA is only present in the cell bodies, this approach does not clarify which subtype might be presynaptic (although the authors propose that it is α3 [26]). Of course, it is also possible that both subtypes have both pre- and post-synaptic localization in brain neurons, as is the case for D2 and D3 receptors for example [30].

To pursue the question of nAChR subtype localization and function in the central nervous system, more specific pharmacological tools are urgently required; the consensus from available data favours the [³H]nicotine binding site (α4β2) as the correlate of presynaptic nAChR on striatal dopaminergic neurons, although the possibility that α3β2 receptors also have a presynaptic role cannot at present be excluded.

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Probing the GABA<sub>A</sub> receptor with antibodies
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Introduction
The inhibitory neurotransmitter γ-aminobutyrate (GABA) elicits its physiological effects through interactions with two distinct classes of cell-surface receptor in the central nervous system. The GABA<sub>A</sub> receptor is a member of the superfamily of ligand-gated ion channels of which the nicotinic acetylcholine and glycine receptors are also members (see [1, 2] for reviews). The GABA<sub>A</sub> receptor, which displays a neuronal distribution and pharmacology distinct from that of the GABA<sub>B</sub> receptor, is G-protein linked [3].

The GABA<sub>A</sub> receptor possesses a complex pharmacology and is regulated allosterically at a number of distinct sites. Thus, GABA or GABA analogues, such as muscimol, regulate the opening and closing of the ion channel on a time scale of milliseconds. Radioligand binding studies have defined at least three other high-affinity binding sites on the receptor complex, namely those for the anxiolytic benzodiazepines, cage-convulsants (e.g. t-butyl bicyclophosphorothioate) and avermectin B<sub>1a</sub> [4]. In addition, the activity of the GABA<sub>A</sub> receptor is also subject to modulation by a variety of hypnotic agents, including depressant barbiturates and anaesthetics [5, 6] and by some endogenous steroids [7–9]. However, in the absence of suitable high-affinity ligands which can be displaced competitively by barbiturates or steroids, the precise nature of the interaction between these drugs and the GABA<sub>A</sub> receptor has remained unclear.

Molecular structure of the GABA<sub>A</sub> receptor
The affinity-purified GABA<sub>A</sub> receptor is a heterooligomeric glycoprotein. Two polypeptides of molecular mass 53000 and 57000 Da (α and β subunits, respectively) were obtained after purification of the bovine receptor [10]. Taguchi & Kuriyama [11] have described two polypeptides (48.5 and 54 kDa) after purification of a Nonidet P-40 extract of rat cerebral cortex and the porcine cerebral cortex receptor appeared to be composed of 49 kDa and 55 kDa polypeptides [12]. The bovine receptor was reported to exist as an α<sub>2</sub>β<sub>2</sub> heterotetramer [13] although four polypeptides were apparently revealed (56, 60, 61 and 66 kDa) in the purified rat brain cortex receptor [14]. More recently, higher resolution gel electrophoresis has revealed that the α and β subunit bands are, in fact, composed of several different polypeptides [15]. Photo-affinity-labelling and molecular cloning studies have also supported the notion that the receptor complex is composed of more than two distinct polypeptides [16–18].

The complete primary sequences of the α and β subunits of the bovine GABA<sub>A</sub> receptor were deduced from the corresponding cDNA sequences [19]. As a result of these studies, several common features of the α and β polypeptides were revealed. The hydropathy profiles predicted both subunits contained four hydrophobic transmembrane...