AUTORADIOGRAPHICAL EVIDENCE OF NICOTINIC RECEPTORS IN RAT BRAIN

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INTRODUCTION

In the peripheral nervous system, nicotine and muscarine mimic different actions of acetylcholine (ACh) and act through different receptors. Peripheral nicotinic receptors fall into two classes (1): C6 type cholinoceptors, found principally at autonomic ganglia, where cholinergic neurotransmission is selectively blocked by hexamethonium; and C10 type cholinoceptors, occurring at the muscle endplate, where decamethonium is a much more potent antagonist. There is less consensus as to the nature of central nicotinic receptors; cholinoceptors with mixed nicotinic and muscarinic properties have been invoked, while others have argued for a noncholinergic receptor for nicotine. What can be said with confidence, however, is that nicotine generally acts through central receptors of some sort. Thus, many biochemical (2), electrophysiological (3), and behavioral (4,5) studies have demonstrated central actions of nicotine that are blocked by nicotinic antagonists, and some of these actions are known to be stereoselective (6,7,8).

RADIOLIGANDS FOR LABELING PUTATIVE NICOTINIC RECEPTORS

Various compounds with known peripheral actions have been employed as radioligands for labeling brain nicotinic receptors. Until recently, the most frequently used was the nicotinic antagonist [¹²⁵I]alpha-bungarotoxin (BTX), which binds to a subunit of the well-characterized nicotinic receptor/ionophore macromolecule of Torpedo californica and also at the mammalian neuromuscular junction. In brain homogenates, BTX binds in a manner consistent with a nicotinic receptor label, insofar as binding is saturable, of high affinity, and selectively inhibited by nicotinic compounds (for review, see 9). Among other peripheral antagonists, nicotinic binding to brain has been reported with radiolabeled d-tubocurarine, naja naja siamensis alpha-toxin, and dihydro-beta-erythroidine, but the extent to which these ligands label the same nicotinic site has not been determined.
In recent years, attention has focused on nicotinic agonists. In 1980, Romano and Goldstein (10) reported the stereospecific and saturable binding of tritiated nicotine to rodent brain homogenates. As subsequently confirmed by other groups (11,12,13), binding was of high affinity (as reflected by a nanomolar dissociation constant), and was potently inhibited by nicotinic agonists including Ach, but not by C6- or C10-selective antagonists. A second, lower-affinity site has also been detected, but this is of doubtful pharmacological significance, since it is of high capacity and possesses little or no regional distribution (10,11,13,14). In contrast, Sloan, Todd, and Martin (15) reported the possible existence of five sites at which l-nicotine may act, including a stereoselective site (KD approx. 5 nM) which may correspond to the high-affinity site previously reported. In another report (16), two sites were described in mouse brain, both with much lower affinities, and the higher-affinity site, which lacked stereoselectivity, was not saturable by l-nicotine in concentrations as high as 100 µM. Schwartz, McGee, and Kellar (17) described the use of [3H]ACh as a ligand for putative nicotinic cholinceptors in brain; in the presence of excess unlabeled displacer and an inhibitor of acetylcholinesterase, [3H]ACh bound to homogenates with characteristics similar to those of the high-affinity [3H]nicotine binding site.

ANATOMICAL DISTRIBUTION OF NICOTINIC BINDING SITES IN RAT BRAIN

There is little agreement as to the regional distribution of high-affinity [3H]nicotine binding determined in tissue homogenates of microdissected brain regions. In particular, variable amounts of binding are attributed to the hypothalamus and hippocampus relative to other brain areas (11,12,14,18,19). In order to obtain a much more detailed map of binding sites, we turned to autoradiography (for details, see ref. 20). For direct comparison with autoradiography, the binding of [3H]d,l-nicotine was characterized and optimized using unfixed slide-mounted sections of rat brain which were transferred into vials for liquid scintillation counting. Displaceable binding, assessed by the addition of excess unlabeled l-nicotine, was enhanced by the addition of calcium chloride and reached equilibrium after 10 to 20 minutes at room temperature. Following incubation with radiolabeled nicotine, sections were washed in ice cold buffer which selectively removed nondisplaceable binding with little loss of displaceable binding. The amount bound was proportional to section thickness (8 - 48 µm). Scatchard analysis of coronal sections taken through the midstriatal forebrain revealed a single-affinity site (Fig. 1). The dissociation constant (3.5 nM) obtained was lower than in most reports employing homogenates, whereas the binding capacity (Bmax) was comparable. As originally described in homogenates (10), binding to tissue sections was selectively inhibited by nicotinic agonists; antagonists selective for either ganglia or muscle endplate were weak inhibitors. The biologically more potent isomer, l-nicotine, was seventeen times more potent than d-nicotine in inhibiting binding of the racemic radioligand; and at the low concentration used for autoradiographic labeling, it is likely that [3H]d-nicotine was binding to a negligible extent. Consistent with this suggestion, we have since obtained KD values of around 1 nM using pure [3H]nicotine instead of the racemic label, and we have found that the two ligands yield autoradiographic patterns which are qualitatively indistinguishable (unpublished observations). Displaceable binding accounted for 90% of total binding across a range of radioligand concentrations (Fig. 1), and the high signal-to-noise ratio was reflected in the autoradiographs which were obtained at 3.5 nM [3H]nicotine. The autoradiographic distribution was discrete and respected anatomical demarcations. Dense labeling was observed in the medial habenula and interpeduncular nucleus, which appear to belong to a common cholinergic system; in the so-called specific motor