RADIOLIGAND LABELLING OF NICOTINIC RECEPTORS IN MAMMALIAN BRAIN

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INTRODUCTION

Nicotine clearly exerts many of its actions in the brain and spinal cord by acting at receptors, a conclusion drawn from biochemical (Chesselet, 1984), electrophysiological (Krnjevic, 1975), and behavioural (Romano et al, 1981; Clarke and Kumar, 1983) studies. Nevertheless, the pharmacological nature of these receptors is still a matter of debate; in particular, the extent of similarity between central and peripheral nicotinic receptors has been questioned. This chapter attempts to categorise the binding sites of nicotinic radioligands in mammalian brain and to examine whether such sites represent pharmacological sites of action for nicotine and acetylcholine.

SIMILARITIES BETWEEN THE CENTRAL AND PERIPHERAL PHARMACOLOGICAL ACTIONS OF NICOTINE

In the peripheral nervous system, two classes of nicotinic cholinoceptors are recognised (Brown, 1979) : C6-type receptors (found principally at autonomic ganglia), so-called because nicotinic cholinergic neurotransmission is selectively blocked by hexamethonium ; and C10-type receptors which occur at the muscle endplate, where decamethonium is much the more potent antagonist. Autonomic ganglia are more sensitive than skeletal muscle to the stimulant and desensitising actions of nicotine (Paton and Savini, 1968), and there appears to be a dose or concentration "window" in which nicotine can alter ganglionic transmission without affecting the muscle endplate. Within this window, nicotine exerts central actions which can be blocked by C6-selective antagonists such as mecamylamine (Chesselet, 1984;

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Clarke and Kumar, 1983, 1984; Clarke et al, 1985a). Moreover, tachyphylaxis or acute tolerance, which occurs readily at ganglia (Paton and Savini, 1968), is commonly associated with the central mecamylamine-sensitive actions of nicotine (see Clarke et al, 1985b). Thus, if central nicotinic receptors resemble their peripheral counterparts, there is ample pharmacological evidence for a C6 type of central receptor.

Evidence for central C10-type receptors is meagre. There are no convincing reports of central mecamylamine-insensitive actions following systemic administration of nicotine. However, systemic doses of nicotine high enough to act at such putative C10 sites would affect neuromuscular function as well. This problem can be circumvented by microinjection or microiontophoresis, and use of these techniques supports the existence of C10-type receptors in certain brain sites (Bradley and Lucy, 1983; Farley et al, 1983; Zatz and Brownstein, 1981).

RADIOLIGANDS FOR CENTRAL NICOTINIC RECEPTORS

Not surprisingly, all radioligands which have been employed to date are compounds with known nicotinic agonist or antagonist actions in the periphery (Table 1). Alpha-bungarotoxin (BTX), until recently the ligand of choice, binds to the mammalian neuromuscular junction, where it blocks nicotinic cholinergic transmission. However, BTX binds to mammalian autonomic ganglia in the absence of functional blockade, and for this and other reasons (Oswald and Freedman, 1981), it is thought that BTX labels some entity other than the nicotinic cholinergic receptor in some species and in some tissues. In rat brain homogenates, ^{125}I-BTX binds with high affinity in a saturable manner and binding is selectively inhibited by nicotinic compounds including nicotine itself (Oswald and Freeman, 1981). Naja naja toxin and BTX share an exclusively neuromuscular action in the mammalian peripheral nervous system, and appear to bind to a common site in brain (Speth et al 1977). Of the other antagonists which have been employed for labelling putative brain nicotinic receptors, d-tubocurarine, and dihydro-beta-erythroidine (DHBE) are both nonselective insofar as they block cholinergic transmission at

muscle and ganglia. Hence it is unlikely that either of these selectively label a "neuromuscular" type of nicotinic receptor in brain (pace Williams and Robinson, 1984), and indeed the regional distribution of DHBE binding is unlike that of BTX (Williams and Robinson, 1984).

Several groups have now described the stereospecific, saturable, and reversible binding of tritiated nicotine to rodent brain homogenates (e.g. Romano and Goldstein, 1980; Marks and Collins, 1982; Costa and Murphy, 1983; Benwell and Balfour, 1985) or to unfixed brain sections (Clarke et al, 1984). Binding is of high affinity (reflected by a nanomolar dissociation constant), and is potently inhibited by nicotinic agonists including acetylcholine (ACh), whereas most C6-selective and all C10-selective antagonists tested to date are ineffective. Binding with the same characteristics occurs in human (Shimohama et al 1985) and monkey brain (P.B.S. Clarke, unpubl). Schwartz et al (1982), employing ^{3}H-ACh in the presence of a cholinesterase inhibitor, obtained closely similar results in rodent brain when muscarinic sites were protected with an excess of atropine.

Some investigators have detected a second site of lower affinity for ^{3}H-nicotine, of doubtful pharmacological significance in view of its high capacity and lack of regional distribution (Romano and Goldstein, 1980; Marks and Collins, 1982; Benwell and Balfour, 1985). More detailed analysis of rat brain homogenates indicates the possible existence of up to five different affinity sites or states for L-nicotine (Sloan et al, 1984), including a high affinity, stereoselective site reminiscent of that reported by others. It is unclear which of these multiple binding sites represent in vivo sites of action of nicotine and/or ACh, and to what extent their detection depends on the method of tissue preparation and incubation conditions employed. In this regard, unfixed cryostat-cut rat brain sections appear to contain a single class of affinity sites for ^{3}H-nicotine (0.5 - 14 nM), as demonstrated by Scatchard analysis (Clarke et al 1984) and also by Hill plot analysis (Figure 1).

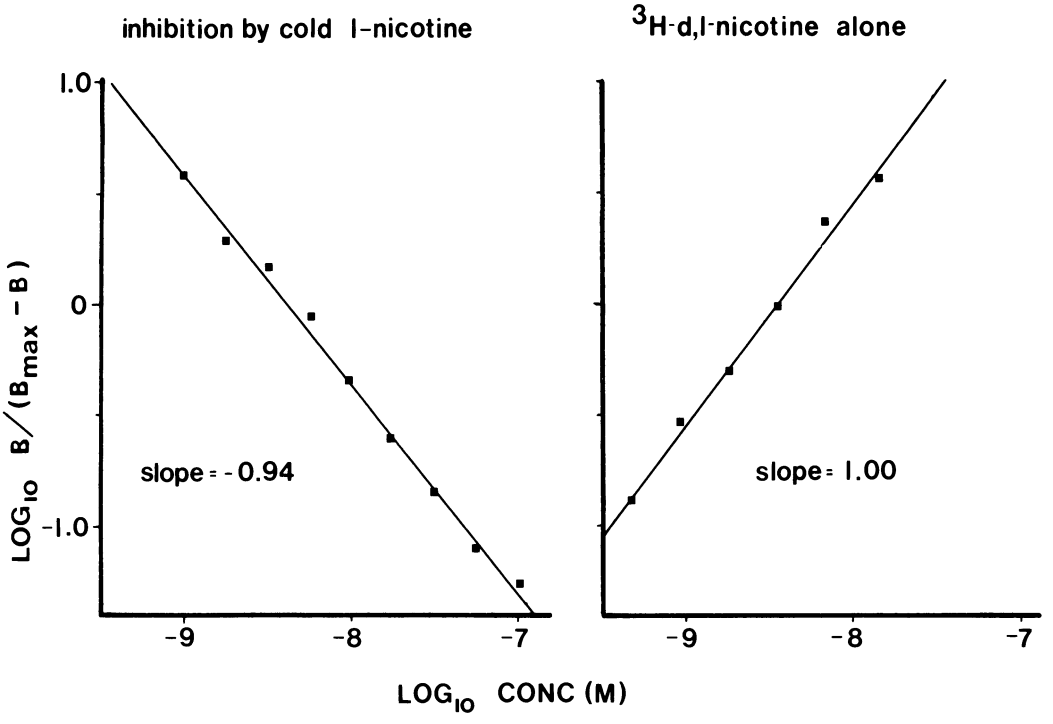

Figure 1. Hill plots of ^{3}H-D,L-nicotine binding to unfixed sections of rat brain (data from Clarke et al, 1984). Left graph: inhibition of specific ^{3}H-nicotine binding by unlabelled L-nicotine. Right graph : specific binding of ^{3}H-nicotine (0.5 - 14 nM) in the absence of inhibitor. Specific binding defined as that displaced by 10 uM L-nicotine.

It is now clear that whilst ^{3}H-nicotine and ^{3}H-ACh probably label the same site in brain, ^{125}I-BTX labels a different nicotinic binding site. Initial studies of homogenate tissue showed that the regional distribution of ^{125}I-BTX binding sites was not correlated with the distribution of either ^{3}H-ACh or ^{3}H-nicotine labelling (Schwartz et al, 1982; Marks and Collins, 1982). In order to clarify the relationship between these three ligands, we undertook an autoradiographic comparison in adjacent brain sections (Clarke et al, 1985b). The autoradiographs showed that ^{3}H-ACh and ^{3}H-nicotine produce seemingly identical patterns of labelling (Figure 2), and no clear differences were observed at any brain level. In contrast, ^{125}I-BTX bound with a strikingly different anatomical distribution. Neither pattern of labelling represented a subset of the other.

^{3}H-ACh ^{3}H-Nic ^{125}I-BTX

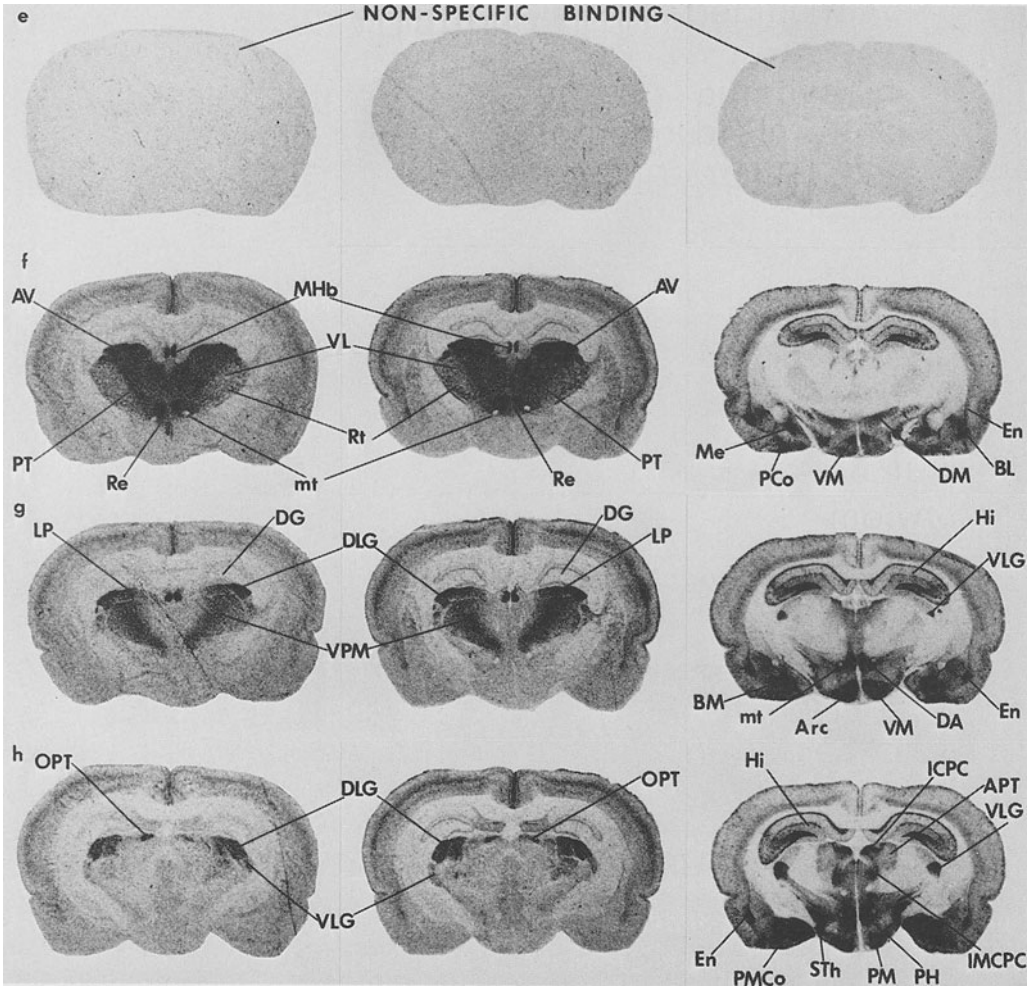

Figure 2. Autoradiographic images showing the distribution of ^{3}H-acetylcholine (left), ^{3}H-nicotine (middle), and ^{125}I-BTX (right) labelling in coronal sections of rat brain (from Clarke et al, 1985b). Nonspecific binding was assessed in the presence of excess L-nicotine.

It should be stressed that ^{3}H-nicotine and ^{3}H-ACh were used in a nanomolar concentration in order to label the high affinity site; only in the micromolar range do nicotine and ACh displace ^{125}I-BTX binding appreciably. Thus it is conceivable that higher concentrations of ^{3}H-nicotine or ^{3}H-ACh would have labelled the ^{125}I-BTX site. Recent biochemical comparisons between ^{3}H-nicotine and ^{3}H-ACh binding support a common molecular site (Martino et al, 1985).

We have proposed that both populations of binding sites may represent in vivo sites of action of nicotine and ACh in the brain (Clarke et al, 1985b). In particular, the tritiated agonists appear to label nicotinic cholinoceptors of the C6 type, whereas sites labelled by ^{125}I-BTX may resemble those at the neuromuscular junction.

EVIDENCE THAT ^{3}H-AGONIST BINDING SITES IN MAMMALIAN BRAIN ARE C6-TYPE NICOTINIC RECEPTORS

(1) High affinity agonist binding is probably neuronal. Thus, it is enriched in the synaptosomal subcellular fraction (Schwartz et al, 1982; Benwell and Balfour, 1985), and lesion studies indicate an association with nerve pathways in the brain (Schwartz et al, 1984; Clarke and Pert, 1985; Clarke et al, 1986).

(2) Chronic nicotine administration to rodents leads to an upregulation of high affinity agonist binding sites in brain (Schwartz and Kellar, 1983; Marks et al, 1983), suggesting that nicotine acts at these sites in vivo.

(3) The pattern of cerebral activation following systemic administration of nicotine in rats has been visualised by the 2-deoxyglucose technique, and is strikingly similar to the anatomical distribution of high affinity agonist binding demonstrated autoradiographically (London et al, 1985). Pretreatment with mecamylamine blocked the effects of nicotine on local cerebral glucose utilisation, implying that putative ganglionic (C6-type) receptors in brain are associated with high affinity binding.

(4) As outlined above, systemic injections of nicotine exert central actions when the drug is given in a dose expected to affect cholinergic transmission in ganglia but not at the neuromuscular junction. Such actions are blocked by the C6-selective antagonist mecamylamine and are typically subject to tachyphylaxis, a phenomenon readily observed at autonomic ganglia.

(5) Tritiated nicotine and ACh bind to mammalian brain with nanomolar dissociation constants, yet exert pharmacological actions in the micromolar range both in vitro and in vivo. Possibly, these ligands bind to a desensitised form of the receptor which has undergone a conformational change resulting in a high affinity for agonists; alternatively, these ligands may bind to a distinct high-affinity site which controls desensitisation but not ion channel opening (cf Conti-Tranconi et al, 1982). Either possibility is consistent with the occurrence of tachyphylaxis to nicotine's central actions.

(6) The inability of most nicotinic antagonists to inhibit high affinity agonist binding could also be accounted for in this way, although some antagonists at least may act on the ion channel rather than on the agonist recognition site (Ascher et al, 1979). It is perhaps significant that neosurugatoxin, which blocks nicotinic cholinergic transmission at autonomic ganglia but not at the neuromuscular junction, potently displaces high affinity agonist binding in brain (Yamada et al, 1985), whereas BTX, which blocks at muscle but not at ganglia, is ineffective (Romano and Goldstein, 1980).

(7) In brain areas which are rich in high affinity agonist binding sites but seemingly devoid of BTX binding (e.g. neostriatum, substantia nigra pars compacta), nicotine's actions are potently blocked by C6-selective antagonists but not by alpha-bungarotoxin (Chesselet, 1984; Clarke et al, 1985a; Rapier et al, 1985).

(8) Agonist-labeled nicotinic receptors may be cholinergic. They fall within cholinergic systems in the brain (Clarke et al, 1984) and are down-regulated by chronic acetylcholinesterase treatment (Costa and Murphy, 1983; Schwartz and Kellar, 1983). Recently, we have identified an excitatory cholinergic input to

dopaminergic neurons of the substantia nigra, which appears to synapse onto nicotinic cholinoceptors labelled by tritiated agonists (Clarke, Pert and Skirboll, in prep).

ARE ^{125}I-ALPHA-BUNGAROTOXIN BINDING SITES IN MAMMALIAN BRAIN C10-TYPE NICOTINIC CHOLINOCEPTORS ?

(1) Ultrastructural and subcellular fractionation studies indicate that much ^{125}I-BTX binding to rodent brain is associated with synapses (Lentz and Chester, 1977; Arimatsu et al, 1978; Hunt and Schmidt, 1978; DeBlas and Mahler, 1978). However, ^{125}I-BTX labels certain areas of rat brain which lack a cholinergic innervation (Hunt and Schmidt, 1979). Thus not all ^{125}I-BTX binding sites represent functional cholinergic receptors.

(2) Binding sites for ^{125}I-BTX upregulate upon chronic treatment with nicotine, but higher infusion doses are required than for upregulation of high affinity agonist binding (Marks, Burch and Collins, 1983). Similarly, in the periphery, higher doses of nicotine are required to induce desensitisation at the neuromuscular junction than at autonomic ganglia (Paton and Savini, 1968).

(3) BTX appears to block the actions of cholinergic agonists in brain areas rich in ^{125}I-BTX binding, such as the suprachiasmatic nucleus of the hypothalamus and inferior colliculus (Zatz and Browstein, 1981; Farley et al, 1983).

(4) The density of hippocampal binding sites for ^{125}I-BTX correlates with susceptibility to seizures induced by high doses of nicotine (Miner et al, 1984).

(5) In brain areas possessing BTX labelling but apparently devoid of high affinity agonist sites (e.g. hippocampus, medulla), microiontophoretic mecamylamine (a C6-selective antagonist) failed to block the effects of nicotine on single unit activity, whereas the nonselective C6/C10 antagonist DHBE was effective (Bradley and Lucy, 1983; Rovira et al, 1983).

(6) The kinetics of ^{125}I-BTX binding to mammalian brain and muscle differ (Oswald and Freeman, 1981). Moreover, antibodies raised against the neuromuscular nicotinic receptor show little

or no cross-reactivity to ^{125}I-BTX binding sites extracted from rat brain (Morley et al, 1983).

(7) At least some ^{125}I-BTX binding sites in mammalian brain may serve a role in the maintenance of synaptic contacts (Oswald and Freeman, 1981). In addition, some sites may represent vestiges from once-functional cholinergic pathways which have disappeared during evolution, of which the retinotectal pathway may be one example.

UNRESOLVED ISSUES

(1) Does the distinction between nicotinic and muscarinic receptors always hold in the CNS ? Outside the electrophysiological domain, a clear dissociation has been achieved between central muscarinic and nicotinic actions, whether in studies of transmitter release, central control of gastric secretion and cardiovascular function, temperature regulation or in the discriminative stimulus properties of drugs. Further evidence is provided by radioligand binding studies (see Clarke and Pert, 1986). However, in certain microiontophoretic electrophysiological experiments, both nicotinic and muscarinic antagonists have been found to block the effects of ACh on neuronal activity, and receptors with "mixed" properties have been invoked (Krnjevic, 1975). In autonomic ganglia, too, this classical distinction sometimes seems to break down, but here the pharmacological ambiguity may reflect an experimental artefact (Volle, 1980). As discussed elsewhere (Clarke and Pert, 1986), a more refined analysis is required in order to exclude the possibility of crosstalk between different receptor subtypes.

(2) Does nicotine act through "non-cholinergic" sites in brain ? This suggestion is based in part on the lack of correlation between the potency of various nicotinic analogues in inhibiting a central pharmacological action of nicotine (prostration) and their ability to inhibit the binding of ^{125}I-BTX or ^{3}H-d-tubocurarine to rat brain membranes (Abood et al, 1981). More recently, however, the prostration syndrome has been prevented by C6-selective antagonists, consistent with its mediation via receptors labeled by tritiated agonists. Taking a

different approach, Rosecrans and Meltzer (1981) reported that the administration of an acetylcholinesterase inhibitor and cholinergic antagonists, in a combination expected to increase nicotinic cholinergic transmission selectively, failed to mimic the centrally-mediated discriminative stimulus action of nicotine in rats. In the absence of dose-response data, it seems premature to propose a noncholinergic site of action for nicotine.

(3) How closely do the results of tritiated agonist binding to brain membranes match those obtained from unfixed brain sections (prepared as for autoradiography) ? Although several protocols have been employed to assess binding to brain homogenates, there is a core of papers indicating a high affinity, stereoselective site possessing high affinity for nicotinic agonists but low affinity for most antagonists (see above). Unfixed brain sections possess a site with similar characteristics (Clarke et al, 1984). However, there is little agreement between studies which have examined the regional distribution of binding in homogenates of microdissected brain tissue; the hippocampus and hypothalamus have yielded particularly variable results. Interestingly, these two regions were almost devoid of binding in autoradiographic studies (Clarke et al, 1984; London et al, 1985). Thus the procedures adopted in autoradiographic studies may have failed to detect a subpopulation of nicotinic cholinoceptors.

(4) If ^{3}H-nicotine and ^{3}H-ACh selectively label C6 receptors, this should be demonstrable in the periphery either in autonomic ganglia or in adrenal medulla. Whilst this test may yet to be carried out, ^{3}H-nicotine does not appear to label muscle nicotinic cholinoceptors under conditions where brain receptors are labelled (P.B.S. Clarke, unpubl.).

(5) Finally, more work is needed to test the possibility of functional C10-type receptors in the brain, especially in areas rich in ^{125}I-BTX binding.

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