Effects of Nicotine on
Biological Systems II
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CURRENT CONTROVERSIES IN NICOTINE RESEARCH

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Summary: Nicotine research has proliferated alarmingly in recent years and the profusion of data makes it hard to maintain a critical eye on developments outside one's immediate speciality. In this brief review, I discuss a number of widely-held views which I believe deserve to be questioned.

Is nicotinic cholinergic transmission an established feature of the mammalian brain ?

Cholinergic pathways in the brain have been elucidated in several mammalian species, most thoroughly in the rat. In this species, transcripts encoding various nicotinic cholinoceptor (nAChR) subunits have been mapped by in situ hybridization histochemistry (e.g. 1). At the protein level, certain nAChRs have also been mapped anatomically by radioligand autoradiography (e.g. 2) sometimes combined with lesions, and by immunohistochemistry (e.g. 3,4). Electron microscopic localization has also been reported using certain probes that recognize nAChRs (e.g. 4). At a functional level, electrophysiological studies have identified many brain nuclei in which neurons are sensitive to the application of nicotinic agonists, and nicotinic agonists have been shown to increase transmitter release in a number of brain areas, via direct actions on nerve terminals (5).

Despite the plethora of anatomical and functional information available on cholinergic pathways and nAChRs, there have been very few attempts to demonstrate sites of nicotinic cholinergic transmission in the mammalian brain. There are several ways to address this question, some yielding results more easily than others, but none easy to interpret. A rather global approach is to examine the effects of chronic in vivo treatment with acetylcholinesterase inhibitors (AChEIs) on radioligand binding to nAChRs. However, despite early findings

that seemed to validate this general approach, results from different studies have been contradictory. This approach is also complicated by difficulties in quantifying receptor density and by uncertainties about what triggers receptor up- or down-regulation (see below), not to mention possible direct actions of AChEIs on nAChRs (e.g. 6). A more direct demonstration of nicotinic cholinergic transmission would involve selective activation of the putative cholinergic input combined with local pharmacological interventions at the putative site of neurotransmission. For several reasons, this is very hard to do in the brain, most notably because to selectively stimulate identified cholinergic pathways is well nigh impossible. Thus, evidence of nicotinic cholinergic transmission in mammalian brain is remarkably slight, being limited to only a few brain nuclei (7).

What are the consequences of CNS nAChR blockade ?

If nicotinic cholinergic transmission is an important feature of brain function, it should be possible to detect behavioural or other changes when nAChRs are blocked. The nicotinic antagonist used in the great majority of behavioural experiments is mecamylamine. Typically, mecamylamine blocks nicotine's CNS actions completely when it is systemically administered in a dose of 1 mg/kg or below. Mecamylamine appears to act insurmountably in the CNS (8), and thus it should not be necessary to administer a higher dose in order to block CNS nicotinic cholinergic transmission. Indeed, it is not clear whether a selective *nicotinic* block can be achieved at doses much above 1 mg/kg, as high concentrations of mecamylamine block NMDA-type glutamate receptors (9). Thus, cognitive and other deficits that are obtained only at high doses of mecamylamine (e.g. 5 or 10 mg/kg sc) do not provide strong evidence for the existence of nicotinic cholinergic transmission in the brain. Although rather low doses of mecamylamine have been shown to impair mental functioning in human subjects, it is not yet clear, I believe, whether these effects are due to a *central* action of the antagonist or whether they derive indirectly from ganglion block.

Are brain nAChRs precisely located across the neuronal surface ?

If acetylcholine (ACh) is rapidly hydrolyzed after release in the brain, one might expect nAChRs to be preferentially located at synapses. However, the limited data available are equivocal on this point (3,4). Moreover, several neural pathways have been identified in

mammalian brain where nicotinic receptors are present both at the level of cell bodies and terminals (see ref. 7). This arrangement suggests that nAChRs, once made, are not precisely transported to particular loci on the cell membrane.

Are α4/ß2-containing nAChRs particularly prevalent in brain ?

Two major problems are encountered in trying to assess the relative prevalence of nAChR subtypes in the brain. First, the number of such subtypes is unknown and potentially large, and selective probes are only available for a few of them; tissue levels of mRNAs encoding different nAChR subunits offer a poor substitute for protein measurements. The second problem lies in the use of radioligands such as ^{3}H-nicotine, ^{3}H-ACh and ^{3}H-cytisine to label nAChRs containing α4 and ß2 subunits (10). The binding of these radioligands appears to rely on a shift in the equilibrium towards a high-affinity state of the receptor. This shift may not be absolute, implying that the B_{max} obtained in binding experiments may only approximate the true density of receptors, a suggestion supported by a report that the measured B_{max} of high-affinity ^{3}H-agonist binding can be increased by the mere addition of drugs to the in vitro binding assay (11).

Do α4/ß2-containing nAChRs mediate actions of smoking doses of nicotine ?

The concentration of nicotine in the brain of human tobacco smokers is likely to be around 1 μM, possibly peaking to 10 μM after a puff is taken. Correlative evidence of several kinds is consistent with the possibility that α4/ß2-containing nAChRs mediate some of these effects. For example, the autoradiographic distribution of ^{3}H-nicotine (2), which labels these receptors (10), is similar to the anatomical pattern of neuronal activation shown by 2-deoxy-glucose uptake after systemic injection of nicotine (12). However, given that we have few markers for other nicotinic receptor subtypes, the considerable overlap that exists in the anatomical distribution of nAChR subunits precludes a clear conclusion. The elevations of ^{3}H-nicotine binding density found in the post mortem brains of smokers (13) indicates that these receptors are targets for nicotine, but does not necessarily imply that they are *activated* during smoking.

Is chronic nicotine administration necessary for the chronic effects of nicotine ?

Some of the behavioural effects of nicotine in animals are lastingly affected by brief, even single pre-exposure to the drug (14,15). In contrast, the up-regulation of high-affinity ^{3}H-agonist binding that occurs in the brain of rodents chronically treated with nicotine has been assumed to require chronic treatment, since it takes several days to appear (16). The experimental designs used to date have not generally discriminated between effects of nicotine pre-exposure that require multiple nicotine pretreatments and those that simply require time to develop after an initial exposure. This issue is given new impetus by the report of an acute effect of nicotine that develops over several days after drug administration (17).

What triggers nicotinic "receptor up-regulation" ?

Chronic treatment with several nicotinic agonists reliably increases the density of high-affinity ^{3}H-agonist and ^{125}I-alpha-bungarotoxin binding sites in rodent brain. It has been suggested that this "paradoxical" up-regulation occurs through a time-averaged antagonistic effect of nicotine. Were some sort of *functional* blockade the stimulus for up-regulation, nicotinic antagonists should also be effective. However, published findings are mixed, and it is not clear whether appropriate doses of mecamylamine were given. Recently, we have shown that chlorisondamine neither mimics nor blocks ^{3}H-nicotine binding up-regulation, despite producing chronic central nicotinic blockade (18). This suggests that functional blockade of these receptors may *not* be the trigger for up-regulation. In addition, our results suggested that up-regulation of ^{125}I-alpha-bungarotoxin binding by nicotine may require receptor *activation*.

Are actions of nicotine in the brain important in tobacco smoking ?

Most of the evidence that *central* actions of nicotine may be important regulators of smoking was provided twenty years ago, by the observation that the centrally-active antagonist mecamylamine increased smoking behaviour whereas the quaternary antagonist pentolinium did not (19). Only recently were the reinforcing effects of nicotine shown to be of central origin in animals self-administering the drug (20). How closely does this paradigm model tobacco smoking in humans ? One should not forget that tobacco smoking is richly

context-dependent in humans. Furthermore, it should be borne in mind that both the anatomical location and the receptor activation and desensitization kinetics of nAChRs may well vary between species. Certain authors have stressed the role that peripheral cues may play in maintaining smoking behaviour (see Rose, this volume), and in this context, it may be as well to recall that nicotine can stimulate neuronal firing in the brain even before it reaches this organ (21).

Why does nicotine replacement therapy not help everyone ?

Nicotine replacement therapy, whether in the form of gum or transdermal patch, only helps a small minority of smokers to quit for periods of a year or more (22). In absolute numbers, this still represents an important medical advance, but one is left wondering why relapse is the rule rather than the exception. The key, I believe, is to see tobacco smoking as an over-learnt behaviour; merely replacing the nicotine does not remove the smoker's history of repeatedly being reinforced for smoking. For this reason, it may be important to consider the development of more selective antagonists (23).

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