Short report

Nicotinic receptor abnormalities in Alzheimer's and Parkinson's diseases

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SUMMARY   The status of cholinergic receptors in dementia is related to the question of potential cholinergic therapy. Whilst muscarinic receptor binding is generally reported to be normal or near normal, findings are reported which indicate substantial reductions of hippocampal nicotinic (high affinity nicotine) binding (occurring in conjunction with decreased choline acetyltransferase) in both Alzheimer's and Parkinson's but not Huntington's diseases. A further indication that nicotinic receptor function may be abnormal in Alzheimer's disease is the extensive loss of an endogenous compound, detected for the first time in human brain, which inhibits normal nicotinic binding. Both receptor binding and the inhibitor are also substantially decreased with increasing age in the normal hippocampus.

In diseases, such as Alzheimer's, associated with extensive reduction in pre-synaptic cholinergic activity[1] the status of cholinergic muscarinic and nicotinic receptors is still uncertain. Most investigations of the muscarinic receptor indicate that binding is unchanged in Alzheimer's disease although reduced total antagonist binding, selective reductions in the M2 site and moderate reductions in both M1 and M2 sites have variously been reported.[2-6] With respect to nicotinic sites, previous measurements of α-bungarotoxin binding in Alzheimer's disease[7,8] can be questioned on the basis of a dissociation between the nicotinic receptor and toxin binding in the CNS.[9] Recent evidence of a close anatomical correlation between the non-muscarinic binding of acetylcholine and high affinity nicotine binding in the brain[10] suggests that nicotine (as opposed to a α-bungarotoxin) can be employed to detect cerebral nicotinic receptors. In one previous investigation of Alzheimer's disease[4] nicotine binding was reported to be decreased in certain subcortical areas but not the cortex, possibly reflecting the technical limitations of using unwashed membranes[4] which may contain a receptor inhibitor (see below). A later report[11] has indicated extensive reductions of both (3H) acetylcholine and (3H) nicotine binding to washed cortical membranes in Alzheimer's disease.

In the present investigation the nicotinic receptor has been quantified using the binding of (3H) nicotine to washed hippocampal membranes, prepared postmortem from clinically and pathologically assessed patients with Alzheimer's and Parkinson's diseases, both manifesting substantial pre-synaptic cholinergic deficits. In addition to the receptor itself, the inhibition by soluble hippocampal extracts of (3H) nicotine binding to normal membranes, indicative of the presence of a "nicotine-like" endogenous ligand (other than acetylcholine),[12] was also measured.

Methods

Cases, matched for age and autopsy delay (table), were selected according to strict clinical and pathological criteria previously outlined[13,14] and included normal subjects (with no neurological or psychiatric disorder), and patients with Alzheimer's disease, Parkinson's disease (both with and without dementia) and Huntington's disease. Numerous cortical senile plaques and neurofibrillary tangles were seen

Table 1   *Nicotinic receptor binding activities in the human hippocampus*

| | Normal | Alzheimer's disease | Parkinson's disease | | Huntington's disease |
| | | | Non-demented | Demented | |
	mean ± SD				
(^{3}H) nicotine binding* (fmol/mg protein)	10·5 ± 4·4	5·2 ± 3·4‖	6·2 ± 3·2§	6·6 ± 1·6‡	8·0 ± 1·6
Nicotine binding inhibitor† (% inhibition at 40 mg/ml)	24·5 ± 12·5	6·6 ± 7·8‖	22·9 ± 14·9	30·6 ± 20·7	31·5 ± 10·6
Choline acetyltransferase (nmol/h/mg protein)	14·9 ± 5·2	3·6 ± 2·7¶	7·9 ± 2·4‖	2·5 ± 3·1¶	16·3 ± 1·3
Number of cases	11	8	7	4	4
Age (yr)	69 ± 10	76 ± 11	73 ± 7	75 ± 8	67 ± 9
Autopsy delay (h)	42 ± 15	39 ± 27	47 ± 28	47 ± 22	21 ± 15

*Scatchard analyses of pooled normal and Alzheimer tissue from the adjacent hippocampal gyrus (hippocampal tissue availability being restricted) indicated that the receptor abnormality in Alzheimer's disease reflects a reduction in B max rather than K_D. B max values, obtained from computer fitting of the curvilinear Scatchard plot to a two-side model, were 0·57 and 32·80 fmol/10 mg tissue in Alzheimer's disease compared with 2·39 and 86·22 fmol/10 mg tissue in the normal (high and low affinity sites, respectively, K_D values for the high affinity site being 1·00 and 1·48 nM in the Alzheimer and normal, respectively).
†For the receptor inhibitor, IC_{50} values for pooled hippocampal extracts were 87 and 31 mg (original weight)/ml in the Alzheimer and normal groups respectively.
‡, §, ‖, ¶: significantly different from the normal (Mann Whitney U test), p < 0·05, 0·02, 0·01 and 0·001.

in only the Alzheimer cases[13] and in the neurologically normal group there were no neuropathological abnormalities.

Membranes were prepared from frozen hippocampal tissue, homogenised in 10% w/v sodium phosphate buffer (10 mM, pH 7·4), centrifuged (40,000 g, 15 min, 4°C) and washed twice, a procedure judged to remove endogenous inhibitor on the basis of unchanged receptor binding beyond the first wash. Nicotinic binding was estimated by re-suspending membranes (10 mg original weight/ml 50 mM Tris buffer, pH 7·4) and incubating for 20 min. at 25 °C with 4 nM L-(N-methyl-^{3}H)-nicotine (New England Nuclear) in the absence and presence of 0·1 mM (−)-nicotine di-(+)-tartrate (to detect non-specific binding).[10] Labelled membranes were separated by ultra-filtration through GF/C filters pre-soaked in 0·1% poly-lysine.[4] Under these conditions specific (^{3}H) nicotine binding ranged from 50–70% of the total binding.

The nicotine binding inhibitor[12] was estimated in the supernatant fraction from the original homogenate by measuring nicotine binding to thalamic membranes (selected for relatively high receptor binding) in the presence and absence of supernatant (the tissue concentration being selected to give under 70% inhibition, on the basis of a linear relation between concentration and inhibition up to 75%).

Choline acetyltransferase activity and protein were estimated in aliquots of the original homogenate, as previously described.[13]

Results

The mean level of (^{3}H) nicotine binding in the hippocampus was within the range of activities previously reported for archicortical and neocortical structures in the human brain postmortem.[4,11] Compared with the normal group, nicotinic receptor binding was, together with choline acetyltransferase, reduced in both Alzheimer's and Parkinson's diseases (the latter including cases with and without dementia) but not in

Huntington's chorea (table). Moreover, assessment of the endogenous nicotine binding inhibitor revealed a significant decrease of this compound in Alzheimer's disease. The inhibition by soluble hippocampal extracts from control and diseased individuals of nicotine binding to normal human thalamic membranes (selected on the basis of the relatively high binding in this area) was substantially (over 70%) reduced in Alzheimer's disease but not the other groups (table). Scatchard and IC50 analyses conducted on pooled samples (hippocampal material being insufficient for individual analyses) indicated (table) that the decrease in both receptor binding and the binding inhibitor in Alzheimer's disease reflected reductions in the number, rather than affinities, of these molecules.

Neither the receptor nor the inhibitor were, as previously reported[4,11] related to the delay between death and autopsy (r = −0·28 and 0·04, respectively, within the normal group) although both were substantially affected by age. Thus, within an extended neurologically normal group, aged between 40 and 90 years, there was a striking and significant decline in both aspects of nicotinic activity with increasing age (fig). In the combined normal, Alzheimer and Parkinson groups (table) there was a significant correlation (r = 0·49, p < 0·01) between receptor binding and enzyme activity.

Discussion

The present observations of a reduction in nicotinic binding in Alzheimer's disease are consistent with one previous report on washed cortical membranes[11] and provide new data suggesting similar reductions occur in Parkinson's disease. The receptor reduction in pa-
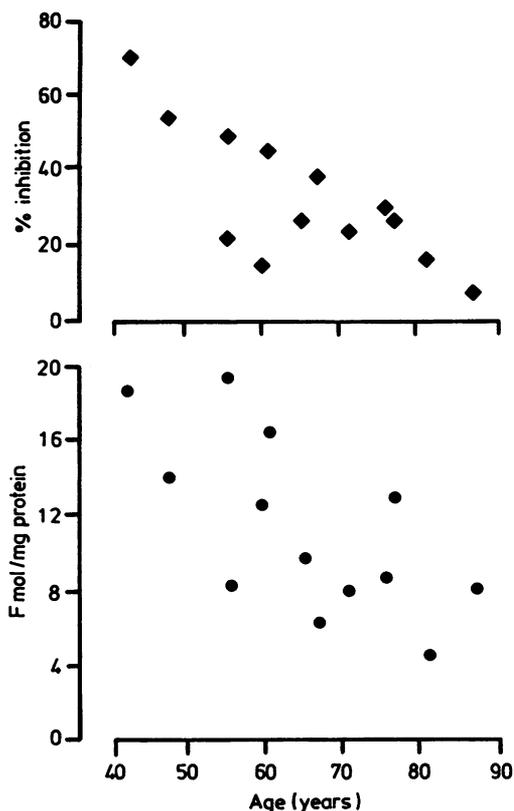
Fig *Decline with age of nicotinic activities in the human hippocampus. Nicotinic binding inhibitor (above, as the percentage inhibition of thalamic receptor binding by soluble extracts at 30 mg/ml) correlates with age (r = −0·783, p < 0·01) as does the receptor binding itself (r = −0·785, p < 0·01). Points represent normal individuals, the group being extended by two cases aged under 50 yrs compared with the control group in the table.*

The reduction in the endogenous nicotinic inhibitor, reported for the first time in Alzheimer's disease, is greater than the receptor abnormality itself (table) and may be more specifically associated with the disease process. It is unlikely that the inhibitor loss is due to non-specific influences such as drug treatment or mode of death since these were similar in the Huntington's disease group. Moreover, preliminary data in Down's syndrome indicate inhibitor reductions in older cases (50-60 yr, with Alzheimer-type pathology) but not a younger (31 yr) case. Together with the normality of the inhibitor in the Alzheimer caudate nucleus (unpublished observation) this suggests a possible association with intrinsic cortical pathology (for example plaques and tangles) not seen in the cases of Parkinson's disease examined, in which the inhibitor was normal (table). The chemical nature of the inhibitor remains to be determined. Our findings agree with a previous report[12] that it is heat resistant and of low molecular weight (under 10,000 daltons). Its anatomical distribution, being relatively concentrated in cortical compared with striatal regions (unpublished observation), suggests it is not acetylcholine or choline.

The status of the cortical nicotinic receptor and particularly the nature of the inhibitor (a putative endogenous modulator of this receptor) should clearly be investigated further in relation to the normal process of aging and in dementing or cognitive disorders such as Alzheimer's and Parkinson's diseases. With respect to normal behaviour, whilst the effects of drugs interacting with the muscarinic cholinergic receptor have been widely investigated (antagonists and agonists generally impairing and enhancing (respectively) memory and information processing) effects of nicotine on such mental functions are less clear. Alterations in arousal[16] and the acquisition of avoidance behaviour[17] in animals have been reported and in man nicotine has the opposite effect to scopolamine (a muscarinic antagonist) in performance involving rapid information processing.[18] Long-term administration of nicotine in experimental animals is reported to increase both the behavioural stimulation effect of nicotine and nicotinic binding of acetylcholine in, amongst other areas, cerebral cortex.[19] If a similar effect were obtained in Alzheimer's disease, then given the low number of binding sites and reduction in a nicotine-like factor in this disorder, nicotine might be worth testing therapeutically.

thological conditions involving cholinergic degeneration (decreased choline acetyltransferase as in Alzheimer's and Parkinson's diseases, table, and Down's syndrome, unpublished observation) but not in those with normal cortical cholinergic activity (Huntington's disease, table, and motoneuron disease, unpublished observation) suggests the receptor is at least partly associated with cholinergic innervation. In the rat,[15] fimbria transection is accompanied by an increase in hippocampal nicotinic (^{3}H) acetylcholine binding which, although inconsistent with the present observations, may reflect a species difference or the relatively non-selective nature of experimental fimbria lesions compared with the pathological condition.

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