Agonist interactions with [^{3}H]-spiperone binding sites on rat corpus striatum membranes

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The development of [^{3}H]-neuroleptics has led to the identification of putative dopamine (DA) receptors on cerebral membranes (Burt, Creese & Snyder, 1976; Howlett & Nahorski, 1978; Titeler, Weinreich, Sinclair & Seeman, 1978). Studies have shown, however, that agonists and antagonists do not bind to the same site in a simple competitive manner (Burt *et al.*, 1976; Titeler *et al.*, 1978). This may reflect the existence of (i) an interconvertible agonist-antagonist receptor site; (ii) the altered availability of binding sites during incubation with agonists; or (iii) an alteration in the affinity of receptors for agonists when coupled to adenylate cyclase. In the present communication we have examined the inhibition of the binding of the [^{3}H]-neuroleptic, spiperone, to rat corpus striatum membranes by dopamine receptor agonists. Furthermore, we have examined whether this inhibition is affected by guanyl nucleotides which have been shown to influence the coupling of several receptors to adenylate cyclase (Maguire, Ross & Gilman, 1977).

Membrane preparations and binding assays were as previously described (Howlett & Nahorski, 1978). In contrast to neuroleptics such as (+)-butaclamol and α-flupenthixol, the agonists DA and apomorphine (APO) displaced [^{3}H]-spiperone binding over three or more orders of drug concentration, producing displacement curves of a 'flattened' nature. Scatchard analysis of these results revealed the possible existence of more than one site for agonists with DA displacement, showing high and low affinity sites (IC_{50}'s of 0.6 and 43 μM) present in approximately equal proportions. APO produced a similar picture (IC_{50}'s of 0.05 and 3.0 μM), although over 60% of the specific binding represented binding to a high affinity site. Antagonists such as (+)-butaclamol and α-flupenthixol, however, only appeared to bind to a single population of high affinity sites.

When GTP was added to the incubation medium, the nucleotide produced a dose related (10^{-6} to 10^{-3}M GTP) shift to the right of the DA displacement curve. The analysis of these results showed a decrease in the affinity for both sites (IC_{50}'s of 4.9 and 220 μM), although the relative proportions of the two sites was unchanged. Other guanyl nucleotides (GPP(NH)P, GDP and GMP) also produced parallel shifts while the actions of non-guanyl nucleotides was much less. Guanyl nucleotides only produced a small parallel shift in the APO displacement of [^{3}H]-spiperone binding (IC_{50}'s of 0.12 and 5.2 μM) and again the proportions of the two sites was not affected. The displacement of [^{3}H]-spiperone binding by antagonists, however, was completely unaffected by any purine nucleotides.

This study emphasizes the complexity of agonist-antagonist interactions at putative DA receptors in the CNS. It seems probable that there are multiple binding sites for dopamine receptor agonists but their relationship to adenylate cyclase remains to be established.

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The relationship between cholinergic axon terminals and α-bungarotoxin binding sites in the rat hippocampus

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Although it is well established that α-bungarotoxin (α-Butx) binds to a component of the nicotinic acetylcholine receptor at the neuromuscular junction (Lee 1972), the nature of the α-Butx binding sites within the central nervous system is less certain. Using a morphological approach we have observed that α-Butx receptors may be found at sites in the rat hippocampus which do not have any apparent cholinergic input and also that during development there is a transient appearance of toxin receptors, again not related in any direct fashion to cholinergic axon terminals.

The distribution of cholinergic axon terminals within the rat hippocampus was determined using autoradiographic procedures 24 h after large injec-

tions of [^{3}H]-proline (40–60 μCi in 0.4 μl) into the medial septal area. α-Butx binding sites were located by incubating fresh cryostat-sections of the rat hippocampus in 10^{-9}M I^{125} α-Butx for 20 min followed by washing and standard autoradiographic preparation. Over 60 rat brains taken from animals whose postnatal age ranged from 1 h to more than 60 days were used in this study.

Comparison of the α-Butx binding pattern with cholinergic axon terminal distribution revealed a fairly close correspondence within the stratum oriens of field CA1 and within the hilus of the dentate gyrus. However there was an intense patch of toxin labelling within an area at the mouth of the dentate gyrus including a portion of field CA3 which was devoid of both cholinergic termination and associated AChE activity. During development an intense patch of α-Butx binding sites was also seen over the pyramidal cell bodies and the adjacent stratum radiatum of field CA1, but this disappeared after the second postnatal week. This area is not thought to be a site of cholinergic termination in either the adult or developing rat brain.

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The binding of choline acetyltransferase to membrane; metabolism of choline in rabbit cortical slices

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The binding of net positively charged (pI, 8.5) rat brain choline acetyltransferase (ChAT, EC.2.3.1.6) to synaptosomal membrane fragments is suggested to reflect distribution *in vivo* mediating the efficient acetylation of choline entering nerve terminals by sodium-dependent high affinity uptake (Atterwill & Prince, 1977, 1978a). Less efficient acetylations in chick brain (Atterwill & Prince, 1978b) and in squid optic lobe (Barker, Dowdall & Mittag, 1975) support this suggestion since ChAT in these species (pI, 5.2 to 6.4) lacks net positive charge (Atterwill & Prince, 1978b; Polsky & Shuster, 1976). Rabbit brain ChAT was known to bind strongly to membrane fragments (Tuček, 1966) and to cation exchange resins, although it also lacked net positive charge (pI, 6.9, Malthe-Sørenssen, 1976). Investigation of the metabolism of [^{3}H]-choline in rabbit brain therefore seemed likely to further clarify the relationship between the molecular properties of ChAT, the affinity of the enzyme for membrane fragments and its disposition *in vivo*.

Metabolism of [^{3}H]-choline in small slices of cortex was investigated as described previously (Atterwill & Prince, 1977; 1978a, b) using samples from male, New Zealand white rabbits (2–2.5 kg). Sodium-dependent uptake (SDU) of choline, calculated by correcting total uptake for that in sodium-free medium (SFU) accounted for 69% total uptake; 91% [^{3}H]-choline accumulated by SDU yielded ACh (Table 1). Acetylation of choline in nerve terminals therefore is apparently as efficient in rabbits as in rats, even though the net charge characteristics of ChAT in these species differ appreciably.

Net charge, therefore, may be an insufficient index of affinity for membrane if, for example, ChAT is asymmetric in charge distribution. The hydrophobic properties recently reported for rat (Rossier, 1977) and bovine brain ChAT (Malthe-Sørenssen, Lea, Fonnum & Eskeland, 1978), however, may be representative of the enzyme from a variety of species, providing an alternative basis for interaction with membrane.

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Table 1 Metabolism of [^{3}H]-choline in rabbit cortical slices

	[^{3}H]-choline uptake		[^{3}H]-label recovered as		
	pmol g^{-1} 10 min^{-1}	% total[b]	[^{3}H]-choline[a]	[^{3}H]-ACh[a]	[^{3}H]-phosphoryl-choline[a]
Total	191.1 ± 7.6[c]	100	36.2 ± 2.4	61.5 ± 2.2	2.3 ± 0.1
SFU	59.0 ± 3.1[c]	30.9	85.0 ± 0.9	8.0 ± 0.8	7.0 ± 0.4
SDU	132.1 ± 7.0[d]	69.1	8.6 ± 3.4	90.9 ± 3.4	0.4 ± 0.1

[a][^{3}H]-metabolite expressed as the mean ± s.e. mean of the average percentages it represented of the total recovered [^{3}H]-label, 4 animals, determinations in triplicate; one animal, determinations in duplicate; 30 mg tissue, 10 min incubation, 37°C.

[b]Percentage of the mean total [^{3}H]-choline uptake.

[c]The mean ± s.e. mean of 14 observations.

[d]The s.e. mean was calculated using the equation: var(x−y) = var(x) + var(y) − 2.covar(x, y) (Colquhoun, 1971).