[^{3}H]Haloperidol and [^{3}H]spiroperidol binding in rat striatum during ageing

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Experimental evidence suggests that neurotransmitters in the brain are modified during ageing (Ordy, Raack & Brizzee, 1975); the synaptic mechanisms of catecholamine, particularly dopaminergic systems, seem to be most affected (Samorajski, 1977).

Dopamine uptake by hypothalamic and striatal synaptosomes, for example, appears to be reduced in senescent mice (Jonec & Finch, 1975), striatal dopamine synthesis is reduced in senescent rats (Samorajski, 1975) and the dopamine content of the caudate nucleus and putamen shows an age-related decrease in man (Carlsson & Winblad, 1976). We have recently shown that the activity of dopamine-stimulated adenylate cyclase, an enzyme postulated to be associated with the dopamine receptor (Kebabian, Petzold & Greengard, 1972), is decreased in the striatum, substantia nigra, nucleus accumbens and tuberculum olfactorium of senescent rats (Govoni, Loddo & others, 1977).

To further investigate dopaminergic receptor function during ageing we have now used the technique of labelling postsynaptic dopamine receptor sites with [^{3}H]haloperidol (Burt, Creese & Snyder, 1976 a, b; Creese, Burt & Snyder, 1975; Seeman, Lee & others, 1976) or with [^{3}H]spiroperidol (Fields, Reisine & Yamamura, 1977). This has provided evidence for specific binding in those brain regions rich in dopaminergic synapses and has offered a new approach to the investigation of the affinity of drugs for the dopamine receptor (Seeman, Chau Wong & others, 1975; Seeman & others, 1976; Creese, Burt & Snyder, 1976).

Healthy mature (about 10 month) or senescent (about 30 month) male Wistar rats were randomly caged to avoid environmental differences and housed at constant temperature and humidity in a light cycle of 12 h. Animal had free access to food and water.

[^{3}H]Haloperidol binding was measured according to Burt & others (1976a) with minor modifications. To each tube containing 1 nM [^{3}H]haloperidol (Sorin Biomedical 8·5 Ci mmol^{-1}) in a volume of 0·2 ml was added 0·8 ml of tissue suspension, prepared as described by Burt & others (1976a), corresponding to 10 mg of tissue. Samples were incubated at 37° for 10 min and then filtered under vacuum through Whatman GF/B filters. Specific binding of [^{3}H]haloperidol was measured as the difference in binding obtained with incubations conducted in the presence or in the absence of 10^{-4} M dopamine. [^{3}H]Spiroperidol binding was measured according to Fields & others (1977) using the ligand at different concentrations (0·1 to 1·2 nM).

Adenylate cyclase activity was measured as described by Carenzi, Gillin & others (1975) using [8-^{14}C]-adenosine triphosphate as substrate. The final incubation mixture of 500 μl contained (mM) trismaleate buffer, pH 7, 5, 80; EGTA, 0·5; $MgSO_4$, 2; theophylline, 10; [^{14}C] ATP (8×10^6 c min^{-1} μmol^{-1}) (0·98 mCi mmol^{-1}) 1, and 150 μg protein of the tissue homogenate. After incubation for 3 min at 30° the reaction was stopped in boiling water for 3 min. Radioactive cyclic 3′,5′-[^{14}C]adenosine monophosphate (cAMP) was separated from radioactive ATP using aluminum and Dowex columns as described by Mao & Guidotti (1974). Protein was measured according to Lowry, Rosebrough & others (1951). [8-^{14}C]Adenosine 5′-triphosphate ammonium salt (specific radioactivity 58 mCi mmol^{-1}) was obtained from the Radiochemical Centre, Amersham.

Our results are summarized in Table 1. The senescent animals showed a significant reduction, about 40%, of specific [^{3}H]haloperidol binding to striatal membranes. Values were 266 and 160 fmol bound mg^{-1} of protein for mature and senescent rats respectively. The stimulation of striatal dopamine sensitive adenylate cyclase induced by dopamine measured in the same groups shows a dramatic reduction in 30 month old rats compared with 10 month old rats confirming our data with 3 months and 24 months old rats of a different strain (Govoni & others, 1978). Protein content was only slightly (about 10%) decreased in senescent animals.

We extended the binding studies to verify if the binding characteristics were the same in both groups of animals and whether the decreased binding observed in senescent rats was due to a decrease in the number of binding sites or to decreased receptor affinity. For this purpose binding experiments with tissue (20 mg) were made using as radioligand at 0·1–1·2 mM for 10 min at 37°, [^{3}H]spiroperidol, a butyrophenone derivative. This

Table 1. *Binding of* [^{3}H]*haloperidol* (*f mol mg*$^{-1}$ *protein*) *and dopamine-stimulated adenylate-cyclase activity* (*pmol cAMP mg*$^{-1}$ *protein min*$^{-1}$) *in striatum of mature and senescent rats.*

	[^{3}H]Haloperidol binding			Adenylate cyclase activity	
	Total	Non spec.	Spec.	Basal	+ Dopamine (50 μM)
Mature	428±37	162±21	266	239±16	486±17
Senescent	288±20*	128± 9	160*	262±21	370±26**
	−33	−21	−40	—	−60

* $P < 0.02$ and ** $P < 0.01$ compared with mature. Results are mean values ± s.e.m. of six separate determinations run in triplicate.

* Correspondence.

has been reported to be clinically more potent than haloperidol (Burt & others, 1976b), and appears to be the more specific ligand than haloperidol for use in the identification of central nervous system dopaminergic receptors (Fields & others, 1977). Experiments were made in the presence or absence of 10^{-4} M dopamine.

The results when presented as Scatchard plots of the specific binding in the striatum of mature and senescent rats, showed that the decreased binding by senescent rats of [^{3}H]haloperidol appears to be confirmed with [^{3}H]spiroperidol. But this appears to be due to a decreased receptor affinity rather than to the total number of binding sites. The densities of receptor were 0·159 and 0·162 pmol mg^{-1} protein for mature and senescent rats respectively. However, the apparent dissociation constant, K_D, for [^{3}H]spiroperidol binding is much larger (0·63 nM) in the striatum of 30 month than in the 10 month animals (0·12 nM). These results clearly support the hypothesis of a decreased dopaminergic receptor affinity with ageing.

The response of dopamine-sensitive adenylate cyclase to dopamine stimulation seems to be influenced more by ageing than is the specific binding of [^{3}H]-haloperidol. This is not surprising since discrepancies between dopamine-sensitive adenylate cyclase activity and dopamine radioreceptor binding kinetics have already been reported. Various dopamine receptor antagonists, for example, show different potencies and affinity constants when tested on dopamine-sensitive adenylate cyclase or on [^{3}H]haloperidol specific binding (Seeman & others, 1975; Creese & others, 1976; Seeman & others, 1976).

These differences do not invalidate our observations in senescent rats but pose the problem of the coupling mechanisms between the dopamine receptor recognition site and the dopamine-stimulated adenylate cyclase.

On the other hand the functional significance in ageing of what would appear to be a generalized deterioration of the dopaminergic receptor remains to be clarified. Cotzias, Miller & others (1974) have shown an increase in the mean life span of mice given dietary doses of levodopa which has been tentatively interpreted as a lesser incidence of intercurrent diseases in test animals than in controls (Cotzias, Miller & others, 1977).

Whatever the final mechanism of levodopa in prolonging the mean life-span of mice it is tempting to suggest that the drug may primarily reinforce the dopaminergic receptor functioning which appears one of the most vulnerable to ageing.

Our findings, with those of Jonec & Finch (1975), Samorajski (1975, 1977); Cotzias & others (1974, 1977) favour the study of the role of the dopaminergic system and the effect of dopamine mimetic drugs on the process of ageing.

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