Binding of [^{3}H]-neuroleptics to dopamine receptors on rat cerebral membranes

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Radiolabelled dopaminergic ligands have been shown to bind to membranes prepared from rat and calf corpus striatum with characteristics indicative of an association with dopamine receptors. Thus [^{3}H]-haloperidol binds with high affinity to a finite number of membrane sites and can be displaced by neuroleptic drugs with relative affinities that match their clinical potencies and, in some cases, their ability to antagonize dopamine-stimulated adenylate cyclase activity (for review see Seeman, 1977). In this communication we have examined the characteristics of binding of [^{3}H]-haloperidol and a new ligand [^{3}H]-spiperone to rat cerebral membranes. Membranes of rat corpus striatum, limbic forebrain, cerebral cortex and cerebellum were prepared by differential centrifugation. The specific binding of both [^{3}H]-ligands to these membranes (binding displaced by 300 μM dopamine, 40–50% for [^{3}H]-haloperidol, 80–90% for [^{3}H]-spiperone) was saturable and of high affinity. The dissociation constants (2.3 nM for [^{3}H]-haloperidol and 0.15 nM for [^{3}H]-spiperone) were almost identical in all cerebral areas, although there were considerable differences in the number of binding sites in the different regions. The binding of either [^{3}H]-haloperidol or [^{3}H]-spiperone had a Bmax (pmol mg protein^{-1}) of 0.51 for striatum, 0.23 for limbic forebrain, 0.20 for cerebral cortex and 0.06 for cerebellum.

Specific [^{3}H]-spiperone (0.6 nM) binding to striatal membranes was stereospecifically displaced by low concentrations of neuroleptics. Thus α-flupenthixol was 50 times more potent than β-flupenthixol (IC_{50} values 4 and 200 nM respectively) and (+)-butaclamol 500 times more potent than (−)-butaclamol (IC_{50}, 4 and 2500 nM). Other neuroleptics also had considerably higher affinities for the binding sites than either the α-adrenoceptor antagonist phentolamine or the β-adrenoceptor antagonist (−)-propranolol. Moreover, dopamine (IC_{50}, 10 μM) and apomorphine (1 μM) were much more potent than noradrenaline (90 μM) or isoprenaline (150 μM). Similar affinities of these compounds were obtained when [^{3}H]-haloperidol (4 nM) was used as a ligand suggesting that both labelled ligands bind to identical sites. The higher affinity and larger specific/non-specific binding ratio of [^{3}H]-spiperone suggests, however, that this drug is the ligand of choice for dopamine receptor binding studies.

Although there is a reasonable correlation between the abilities of drugs to displace [^{3}H]-neuroleptic binding and to inhibit dopamine-sensitive adenylate cyclase, a number of marked discrepancies do occur particularly with the butyrophenones (Seeman, 1977). Moreover, in the present experiments, many neuroleptics, other than butyrophenones, at concentrations 100–1000 fold greater than those that totally occupy [^{3}H]-spiperone binding sites, still failed to antagonise dopamine-stimulated cyclic AMP production in small slices of rat striatum. We are at present investigating these discrepancies and their relevance to the mode of action of neuroleptics.

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Drug induced supersensitivity and choline acetyltransferase (ChA) activity in the rat striatum

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Neuroleptic drugs induce a dopamine receptor blockade and this has been postulated as their therapeutic action (Snyder, Banerjee, Yamamura & Greenberg, 1974). Their prolonged use in man leads to the development of dyskinetic phenomena known as tardive dyskinesia which has been postulated to be due to receptor supersensitivity (Tansy & Baldessarini, 1976). These experiments examine the choline acetyltransferase (ChA) activity of cholinergic neurons in the striatum which are thought to be influenced by dopamine (DA) receptor activity (Agid, Guyenet, Glowinski, Beaujouan & Javoy, 1975), to determine the long-term changes induced by chronic haloperidol administration.

Twelve groups of rats were used to study the ChA activity in the striatum after haloperidol administration given either acutely or chronically. The activity on chronic treatment was measured during the treatment and after withdrawal. The effects of acute administration of L-DOPA on the activity of striatal ChA was examined in control and in animals treated with haloperidol on the various regimes.

Acute administration of the combination of L-

Table 1 The effect of acute and chronic administration of haloperidol on ChA activity in striatum and the additional response to L-DOPA and Ro-4-4602/1

Choline acetyltransferase activity
(Acetycholine synthesized, nmol mg protein^{-1} h^{-1})

Group	*Treatment*	*Pretreatments*		
		Acute[1]	*Chronic (continuously)*[2]	*Chronic (on withdrawal)*[3]
Control	0.9% NaCl	6.82 ± 0.73 (5)*	7.33 ± 1.22 (6)	8.13 ± 1.49 (8)
	L-DOPA + Ro-4-4602/1[4]	9.1 ± 1.47 (5) §	10.36 ± 1.94 (5)	8.23 ± 2.58 (10)
Haloperidol	0.9% NaCl	4.64 ± 0.9 (5) §	8.72 ± 1.18 (5)†	12.40 ± 1.25† (5)
	L-DOPA + Ro-4-4602/1	5.14 ± 0.39 (5)	8.61 ± 2.43 (5)	17.76 ± 3.21† (5)

All drugs dissolved in 0.9% sodium chloride solution
1. Haloperidol (1.5 mg/kg i.p.) and assayed 2.5 h later
2. Haloperidol (1.5 mg/kg) daily for 12 days and assayed 2.5 h after the last dose
3. Haloperidol (1.5 mg/kg) daily for 12 days and assayed 48 h after the last dose
4. L-DOPA (50 mg/kg i.p.) given 30 min after Ro-4-4602/1 (50 mg/kg i.p.) and assayed 30 min later. All control animals received corresponding volumes of 0.9% sodium chloride at appropriate times

ChA was assayed according to Fonnum (1975)
* mean ± s.d. (number of observations)
§ $P < 0.025$ compared with controls (Student's t-test)
† $P < 0.005$ compared with controls (Student's t-test)

DOPA and the decarboxylase inhibitor Ro-4-4602/1 which gives behavioural evidence of increased central dopaminergic activity (Butcher & Engel, 1969) increased ChA activity significantly ($P<0.025$) (Table 1). Conversely acute administration of haloperidol, a dopamine receptor blocker, caused a significant fall in ChA ($P<0.01$). After a single dose of haloperidol, treatment with L-DOPA and RO-4-4602/1 no longer increased ChA when compared with haloperidol alone suggesting that it does not overcome the DA blockade produced by haloperidol. On the other hand after chronic administration of haloperidol ChA rose, although not significantly above the levels seen in controls; it was however higher than the activity seen in acute treatment ($P<0.001$). Again, as with acute haloperidol treatment, acute treatment with the combination L-DOPA and Ro-4-4602/1 failed to change the ChA activity.

In the chronically treated animals from which haloperidol was withdrawn for two days there was an increase in the activity of ChA when compared with controls ($P<0.001$) and also with similar animals in which the haloperidol had not been discontinued ($P<0.005$). This activity was highest ($P<0.001$) when L-DOPA and Ro-4-4602/1 were given on haloperidol withdrawal.

If ChA activity is an index of the firing of the cholinergic neuron in the striatum then we could conclude that dopamine causes excitation of cholinergic interneurons. It would also seem that supersensitivity after withdrawal of haloperidol is associated with the high activity of ChA and that this activity is accentuated on dopamine receptor stimulation.

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