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Dopamine receptor changes after long-term haloperidol treatment in rats

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Tardive dyskinesia is a neurological syndrome associated with prolonged neuroleptic treatment of schizophrenic patients (Crane 1968; Faurbye 1970; Faurbye et al 1964). It has been suggested that tardive dyskinesia results from chemical denervation of central dopamine neurons and subsequent development of supersensitivity of the postsynaptic receptor (Rubovits & Klawans 1972). Experiments based on animal models of tardive dyskinesia support this hypothesis. Chronic neuroleptic treatment increases postsynaptic dopamine receptor sensitivity when measured either behaviourally or biochemically (Christensen et al 1976; Klawans & Rubovits 1972; Moore & Thornburg 1975; Sayers et al 1975; Tarsy & Baldessarini 1974; Von Voigtlander et al 1975). The molecular basis for this behavioural change has been reported to be an increase in the number of receptor sites with no change in their affinity as measured by ^{3}H-neuroleptic binding to striatal membrane homogenates (Burt et al 1977; Klawans et al 1977; Muller & Seeman 1977). Previous biochemical reports of the development of model tardive dyskinesia after neuroleptic treatment of animals have generally used treatment periods of 3 weeks or less (Burt et al 1977; Klawans et al 1977; Muller & Seeman 1977). Only one report treated animals for longer periods (Clow et al 1978). In the present experiments we studied the kinetics of [^{3}H]spiroperidol binding to rat caudate nucleus homogenates after 3 and 10 weeks of haloperidol treatment.

Rat food containing 0·01% haloperidol was prepared by grinding regular rat pellets to a fine powder and thoroughly mixing with drug. Control rats received the same powdered food without haloperidol. Male Sabra strain were used in all experiments and weight gain on this diet was normal. The approximate daily oral dose was 3 mg of haloperidol per rat.

Rats were killed 4 days after cessation of haloperidol feeding and the striatum was dissected and stored at —70 °C until assayed. The binding of [^{3}H]spiroperidol to striatal homogenate was determined as described by Burt et al (1976). Striatum was homogenized using a glass-teflon homogenizer in 100 volumes of 50 mM Tris buffer pH 7·7 containing the following components: (mM) NaCl 120, KCl 5, $CaCl_2$ 2, $MgCl_2$, 0·1% ascorbic acid and 10 μM pargyline. The membranes were collected by centrifugation (50 000 g for 10 min) and resuspended in 285 volumes (original wet weight) of buffer. The reaction mixture contained 800 μl membrane suspension, 100 μl [^{3}H]spiroperidol (NEN, 23 Ci mM^{-1}) from 0·1 to 1·0 nM (5 different concentrations) and either 100 μl 0·1% ascorbic acid or 10 μM dopamine (blank) in 0·1% ascorbic acid. After 10 min incubation at 37 °C the reaction was stopped by rapidly filtering through Whatman GF/B glass fibre filters and washing with ice-cold buffer (2 × 10 ml). The filters were counted in 10 cc Instagel after shaking vigorously for 2 h. Specific binding of [^{3}H]spiroperidol was calculated as the number of counts in excess over the dopamine blank. The number of receptor sites and the K_D was determined by Scatchard plot (Scatchard 1949) for each individual rat striatum. Plots were fitted with a standard computer program.

Table 1 shows the change in number of [^{3}H]spiroperidol binding sites after 3 and 10 weeks of neuroleptic treatment. After 10 weeks there is a mean 128% increase in the number of binding sites compared with a mean 59% increase after 3 weeks, suggesting a trend towards increased number of receptors with increased exposure to haloperidol. Analysis of the data of Table 1 reveals a significantly increased variance ($F = 9{\cdot}31$, $P < 0{\cdot}05$) in the number of receptor binding sites after

Table 1. The effect of chronic haloperidol on the number of [^{3}H]spiroperidol binding sites in striatum

	No of receptors (pmol g^{-1} tissue)		
	Control	Haloperidol	% change
3 weeks	32·27 ± 2·30 (±s.e.m., n = 5)	51·30 ± 3·95 (±s.e.m., n = 5)	+58·9*
10 weeks	26·51 ± 2·35 (±s.e.m., n = 6)	60·56 ± 7·87 (±s.e.m., n = 5)	+128**

* $t = 2{\cdot}81$ Student's t, $P < 0{\cdot}05$.
** $t = 3{\cdot}04$, $P < 0{\cdot}02$.
Numbers in parentheses are the number of individual rat striatum assayed.
Behavioural supersensitivity as measured by amphetamine (5 mg kg^{-1})-induced stereotypy developed in a parallel group of haloperidol-fed animals ($t = 4{\cdot}94$ Student's t, $P < 0{\cdot}01$).

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Table 2. The effect of chronic haloperidol on the dissociation constant (K_D) of [^{3}H]spiroperidol to rat striatal membranes

	K_D (nM) mean ± s.e.m. control	haloperidol	% change
3 weeks	0·41 ± 0·07 (n = 5)	0·52 ± 0·05 (n = 5)	+26·8*
10 weeks	0·55 ± 0·05 (n = 6)	1·41 ± 0·24 (n = 5)	+156**

* n.s.
** $t = 2·61$ Student's t, $P < 0·05$.
Numbers in parentheses are number of individual rat striatum assayed.

10 weeks of haloperidol treatment compared with control-treated animals. After only 3 weeks haloperidol there is no significant difference between the variance of the drug and control-treated animals ($F = 2·94$, n.s.).

Table 2 shows the calculated dissociation constant (K_D) for the binding of [^{3}H]spiroperidol to striatal dopamine receptors. As previously reported (Burt et al 1977) there is no change in the K_D after 3 weeks of neuroleptic treatment. After 10 weeks there is a significant increase (156%) in the K_D for the haloperidol-treated rats.

These results confirm previous reports that 3 weeks of haloperidol treatment leads to a significant increase in the number of receptor binding sites (Burt et al 1977; Clow et al 1978; Klawans et al 1977; Muller & Seeman 1977). The increase is more marked after 10 weeks of haloperidol treatment and is accompanied by a significant increase in the variance of the number of receptor sites. Thus some individuals of the genetically hetereogenous rat strain develop a 400% increase over the mean number of dopamine receptors in the control group. This heterogeneity in individual response to neuroleptic treatment may be similar to the clinical situation in tardive dyskinesia, which develops in only a fraction of treated patients. The significant increase in K_D after 10 weeks of haloperidol treatment is the first report of a significant change in receptor affinity and appears to occur only after prolonged neuroleptic treatment and not after shorter time periods. The significance of the K_D change as opposed to the increased receptor number for the model of tardive dyskinesia is at present obscure. Oral feeding of haloperidol to animals, however, makes possible long-term feeding experiments that more closely parallel the human disorder than the 3 week experiments common until now.

January 8, 1979

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