^{3}H-Spiroperidol binding to striatal membranes of mutant Han-Wistar rats which exhibit spastic paresis

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Summary. The binding of ^{3}H-spiroperidol to striatal membranes from a strain of mutant Han-Wistar rats was compared with that in normal littermate animals. The specific binding was less in the mutants than the controls. Scatchard analysis revealed that the K_D- and B_{max}-values for the high affinity binding sites in the mutants are greater than for those in the controls. These findings indicate that the dopamine receptors of the mutants are affected and could explain some of the previous data; it has been suggested that some of the spasticity observed in the mutants may be due to an abnormal functioning of their dopaminergic neurones.

A strain of Han-Wistar rats characterized by progressive spastic paresis and partial paralysis has recently been described[3]. L-DOPA treatment temporarily reverses the symptoms of the mutants but not D-DOPA[4,5]. This observation was instrumental in suggesting that the animals might serve as a model for studying Parkinson's disease. However, the dopamine content in the mutant striata is higher, and not lower than that in controls[4,6]. Furthermore, striatal tissues of the mutants and control littermates have similar capacities to accumulate and release radioactive dopamine[5]. In an attempt to interpret the known data, the dopamine antagonist ^{3}H-spiroperidol was used to compare biochemically the binding characteristics in striatal tissue from mutant and control littermates.

Mutant (homozygous with respect to the gene mutation) and littermate control rats of the Han-Wistar strain were obtained from the Zentralinstitut für Versuchstiere, Hannover. Striata were dissected and usually kept in the deep-freeze for about 3 weeks before use. Frozen striata were subsequently homogenized (1 g/10 ml) in cold 0.05 M Tris-HCl buffer pH 7.4, using a Teflon pestle and glass grinder rotating at 900 rev/min with 8 up and down strokes. After centrifuging the homogenate at about 1000 × g for 10 min, the supernatant was centrifuged at 25,000 × g for 25 min. The resultant pellet was resuspended in the original volume of Tris-HCl buffer and incubated at 37 °C for 20 min in order to reduce any excess endogenous dopamine through metabolism. Thereafter it was centrifuged at 25,000 × g for 25 min and the pellet was resuspended in 4 times the original volume of Tris-HCl and immediately assayed. Usually, 400 µl of the membrane preparation was incubated in 0.05 M Tris/HCl buffer with various concentration of ^{3}H-spiroperidol (New England Nuclear, sp. act. 25.7 Ci/mmole) in the absence (total binding) or presence (nonspecific binding) of 10^{-5} M haloperidol. The final volume was 550 µl. After incubation at 37 °C for 10 min, 500 µl of the sample was rapidly filtered through Whatman GF/B glass fibre filters and washed on the filter with 2 × 5 ml ice-cold 0.05 Tris/HCl buffer. Filters were placed in scintillation fluid and counted at an efficiency of 25%.

The amount of nonspecific binding was the same for both mutant and control striatal membranes and amounted to 30% of the total binding. The specific binding was more or less the same for the mutants and controls when very low amounts of ligand were used (less than 0.7 nM), but at higher concentrations of ^{3}H-spiroperidol (more than 1 nM) the binding gradually increased in the control as compared with mutants. This was reflected in the slope of the initial part of the binding isotherm. The amount of binding is related to the wet weight of tissue, rather than protein content, because this proved to be most convenient. We were also aware that differences in the protein content between mutants and controls might exist.

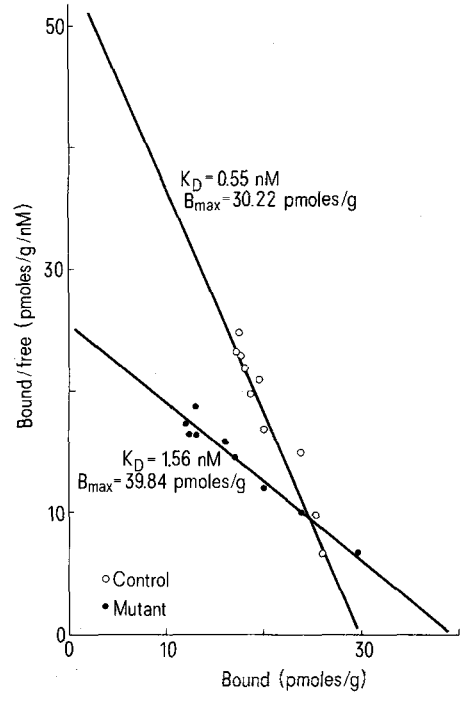
Scatchard analysis of the binding of 15 different concentrations of ^{3}H-spiroperidol to striatal membranes of control and mutant rats (ranging between 0.7 and 50 nM) was carried out in 2 separate experiments. 2 binding sites could

Affinity constants for the binding of ^{3}H-spiroperidol to striatal membranes of mutant and control littermates

	Control animals		Mutant animals	
	K_D (nM)	B_{max} (pmole/g)	K_D (nM)	B_{max} (pmole/g)
High affinity	0.55[a]	30.22[b]	1.56[a]	39.84[b]
Low affinity	79.36[c]	1233.54[d]	118.57[c]	1444.25[d]

Values deduced from Scatchard plots. Results are the mean of 5 separate experiments for the high affinity constants and 2 separate experiments for the low affinity constants, each being performed in triplicate. Variation between the values in individual experiments was between 10 and 14%.

[a,b] Significantly different by Student's t-test $p < 0.05$; [c,d] no difference.



Scatchard analysis to show the high affinity binding sites of ^{3}H-spiroperidol to striatal membranes from mutant and control littermates. It can be seen that the K_D- and B_{max}-values for the high affinity binding sites are higher in the mutants than in the controls. Results are the mean of 5 different experiments performed in triplicate. Variation between individual values in single experiments was between 10 and 14%.

be identified in tissue derived from control and from mutant animals. The K_D- and B_{max}-values for both high and low affinity binding were different (table). Since the high affinity binding sites are thought to reflect the characteristics of the true physiological receptors, Scatchard analysis of the high affinity binding sites was repeated on a further 3 occasions (fig.). The high affinity K_D- and B_{max}-values for the control animals are significantly lower (K_D= 0.55 nM, B_{max}=30.22 pmole/g) than the mutants (K_D= 1.56 nM, B_{max}=39.84 pmole/g), and the K_D-value observed for the controls is similar to that reported by Pedigo et al.[7]. The present results clearly show that differences exist in the binding properties of ^{3}H-spiroperidol to membranes derived from striatal tissue of mutant and control littermate rats. We interpret the data as indicating that the dopamine receptors of the mutant animals are affected, which may be the case of the differences found between the dopamine content of control and mutant animals, despite the fact that the tyrosine-hydroxylase activity in the mutant striatum is lower than in the controls[4,5]. It is, however, acknowledged that ^{3}H-spiroperidol also binds to serotonin receptors[8] and that the serotonin content in the striatum of mutant animals is greater than in the controls[5]. Serotonin was, nevertheless, found to compete with ^{3}H-spiroperidol binding sites to the same extent in both tissues (results not shown). This strengthens the idea that the differences in the binding properties of ^{3}H-spiroperidol are due to an alteration in the dopamine receptors of the mutants. Experiments are now in progress using the dopamine specific ligand ^{3}H-ADTN to corroborate the conclusions made in this study.

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The acute effects of sulpiride on the central dopamine turnover in rats: a quantitative histochemical study

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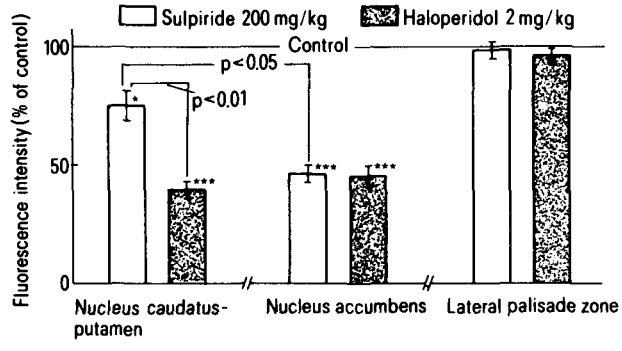
Summary. Sulpiride accelerated the dopamine turnover preferentially in the mesolimbic as compared to the nigrostriatal dopamine system. However, the tuberoinfundibular dopamine turnover was not affected by sulpiride or haloperidol.

The pharmacological spectrum of sulpiride is widely different from that of other neuroleptics. In animals, sulpiride does not possess typical neuroleptic properties, whereas this drug is clinically characterized by its antipsychotic action causing few extrapyramidal symptoms[2]. It was reported, using the chemicalanalytical technique, that sulpiride accelerated preferentially the mesolimbic dopamine (DA) turnover[3,4]. The present experiment was carried out, using quantitative histochemistry[5], in order to confirm former biochemical findings, and to examine the action of sulpiride on the DA turnover in the median eminence. Haloperidol was used for reasons of comparison with sulpiride.

Material and methods. Male Wistar rats (180–250 g) were used in this investigation. The DA fluorescence intensity was measured by means of quantitative microfluorimetry[5] applied to the Falck-Hillarp histofluorescence method[6]. The effect of DL-α-methyltyrosine methyl ester hydrochloride (α-MT, 250 mg/kg) on the DA fluorescence in the brain was examined. Saline and α-MT were administered i.p. 4 h before sacrifice. Sulpiride and haloperidol were administered i.p. 1 h before α-MT in doses of 200 mg/kg and 2 mg/kg, respectively. Control rats were treated with saline in the same way as the neuroleptic-treated rats. All rats were decapitated 4 h after α-MT treatment and the brains were dissected and frozen in isopentane cooled by liquid nitrogen. After formaldehyde gas treatment at 80 °C for 1 h, frontal sections of 10 µm thickness were made according to the atlas of König and Klippel[7]. The DA fluorescence in the following DA terminals was measured by means of quantitative microfluorimetry; dorsal part of the nucleus caudatus-putamen (CP), nucleus accumbens (ACB) at the level of A8920, and the lateral palisade zone (LPZ) in the central region of the median eminence[8]. For measurement of the fluorescence, a microspectrophotometer (Zeiss, MPM-01 system) with a 100-W high pressure mercury lamp, a BP-405/8 excitation filter and a FT-425 dichroic mirror was employed. A LP-450 interference filter was placed between the measuring field and the photomultiplier. The signal from it was led to a digital display unit for recording the fluorescence. The measuring circle had diameters of 2–20 µm. Fluorescence was measured in 50–100 circular areas in the above mentioned regions.



Effects of sulpiride and haloperidol on the dopamine fluorescence disappearance after α-MT. The values are mean ± SEM as a percentage of respective control. * $p<0.01$, *** $p<0.001$ vs respective control; n=4–6.