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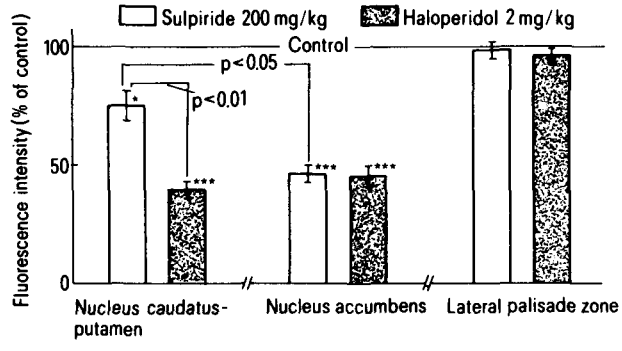
Summary. Sulpiride accelerated the dopamine turnover preferentially in the mesolimbic as compared to the nigrostriatal dopamine system. However, the tuberoinfundibular dopamine turnover was not affected by sulpiride or haloperidol.

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.

Background fluorescence was obtained by measuring tissue fluorescence in the region of the anterior commissure and the ventromedial nucleus which showed no catecholamine fluorescence. Then, net DA fluorescence was obtained by subtraction of background fluorescence. Results were analysed by Student's t-test.

Results and discussion. After treatment with α-MT alone, the DA fluorescence intensities in the CP, ACB and LPZ were 57.16±3.00%, 52.68±2.26% and 77.86±5.05% of control, respectively. The fluorescence reductions of these regions were significant (CP: $p < 0.001$, ACB: $p < 0.001$ and LPZ: $p < 0.01$).

The results after treatment with neuroleptics are shown in the figure. In ACB, which is innervated by the mesolimbic DA system, the acceleration of DA fluorescence disappearance was identical in sulpiride and haloperidol treated rats. However, in CP, which is innervated by the nigro-striatal DA system, the fluorescence intensities in sulpiride- and haloperidol-treated rats were 74.99±6.35% and 39.16±3.45% of the control values, respectively, and the difference between them was significant ($p < 0.01$). Thus, 2 mg/kg of haloperidol accelerated the DA turnover more than 200 mg/kg of sulpiride in the nigrostriatal DA system. In addition, DA fluorescence disappearance after sulpiride was significantly greater in ACB than in CP ($p < 0.05$). Accordingly, sulpiride increased the DA turnover preferentially in the mesolimbic system. On the other hand, haloperidol had a similar effect on both nigro-striatal and mesolimbic DA turnover. In LPZ, which is the terminal region of the tubero-infundibular DA system, no reduction of the fluorescence was observed under these conditions.

The present histochemical results support the previous biochemical findings and suggest that an acute treatment with neuroleptic agents would have little effect on the DA turnover in the median eminence of the rats.

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Decrease in [^{3}H]ouabain binding sites in heart and brain from spontaneously hypertensive rats

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Summary. A decrease in the number of binding sites (B_{max}) for [^{3}H]ouabain was observed in the heart (37%) and the brain (22%) of spontaneously hypertensive rats (SHR) when compared with age-matched control Wistar Kyoto rats. No variation was detected in the affinity constant (K_D).

In several types of experimental volume expanded hypertension (reno-prival, renal insufficiency, mineralocorticoid hypertensions), the sodium potassium pump activity, estimated through the Mg^{++} dependent Na^+, K^+ ATPase (E.C. 3.6.1.3), is decreased in arteries, veins and myocardium (for reviews see Haddy et al.[1], Overbeck[2]). In spontaneously hypertensive rats (SHR) whose genetic hypertension could be of central origin[3] the specific activity of brain ATPase was high; so we compared the Na^+, K^+ ATPase of the Okamoto Aoki strain of SHR to their normotensive controls (Wistar Kyoto rats, WKY) by measuring the [^{3}H]ouabain binding to brain and heart preparations from the 2 consanguineous strains. This technical choice is supported by the fact that there is a specific and stoichiometric ATP dependent binding in brain and heart rat preparations[4,5].

[^{3}H]ouabain (19.3 Ci/mmole) was purchased from New England Nuclear. Male 16-week-old WKY and SH rats, in which hypertension is established, were obtained from Charles River Co. After decapitation brains and hearts were homogenized in 0.32 M sucrose (1:10 w/v and 1:7 w/v respectively). The 49,000×g pellet was diluted in 50 mM Tris HCl buffer pH 7.5 containing 250 mM NaCl, 4 mM $MgCl_2$, 4 mM ATP for brain (40 µg/ml protein) or 20 mM Tris HCl buffer pH 8 containing 150 mM NaCl, 4 mM $MgCl_2$, 4 mM ATP for heart (1 mg/ml protein) and incubated with [^{3}H]ouabain. The reactions were stopped after they had reached their maximum, i.e. 60 min for brain

Binding of [^{3}H]ouabain to brain and heart membranes of spontaneously hypertensive (SH) and normotensive Wistar Kyoto (WKY) rats

	Brain		Heart	
	K_D (nM)	B_{max} (pmoles/mg protein)	K_D (nM)	B_{max} (pmoles/mg protein)
SHR	14.2±0.4	44.6±1.9*	57.2±9.2	0.455±0.036**
WKY	14 ±0.3	57.1±3.3	50.5±5.4	0.722±0.068

K_D and B_{max} were determined from Scatchard analysis. Each value is expressed as the mean±SEM of 5 experiments. Each curve is determined by using 7 [^{3}H]ouabain concentrations from 1 to 80 nM for the brain and from 10 to 200 nM for the heart. The rats were 16 weeks old. Systolic blood pressure is 212±6 mm Hg for SHR and 138±3 mm Hg for WKY rats. *$p < 0.02$; **$p < 0.01$.