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The actions of antipsychotic drugs on dopamine receptors in the rat substantia nigra

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1 The activity of neurones in the zona compacta of the rat substantia nigra was recorded extracellularly *in vitro*. Dopamine produced a dose-dependent depression of firing, threshold doses being in the 3 μM range.

2 The inhibitory effects of dopamine were antagonized by (−)-sulpiride (pA_2 7.5), haloperidol (pA_2 8.4) and cis-flupenthixol (pA_2 6.9). The actions of γ-aminobutyric acid (GABA) were not affected by these compounds.

3 The gradients of Schild plots of data for (−)-sulpiride were less than unity while those for haloperidol and cis-flupenthixol were greater than unity, which suggests that the antagonism was not competitive. This is discussed with regard to the use of a bioassay system in the analysis of the effects of antagonists.

4 Haloperidol and (−)-sulpiride were found to have similar potencies, as dopamine receptor antagonists, to those predicted from biochemical and clinical efficacy studies, but cis-flupenthixol was less potent than expected.

Introduction

Locally-applied dopamine inhibits the firing of identified dopamine neurones in rat brain (Aghajanian & Bunney, 1973; Guyenet & Aghajanian, 1978; Grace & Bunney, 1980). The dopamine receptors are located on the cell bodies, dendrites and axon terminals of the ascending nigrostriatal and mesolimbic dopamine pathways. While the function of these receptors remains unclear, the profound actions of systemic administration of dopamine agonists and antagonists on neuronal firing (Bunney *et al.*, 1973), have clinical significance in the treatment of both schizophrenia and Parkinson's disease.

It has been suggested that the binding of [^{3}H]-spiperone and the inhibition of [^{3}H]-dopamine release in the striatum are valid *in vitro* models for dopamine autoreceptors (Lehman *et al.*, 1983). Recently a substantia nigra slice preparation has been described, in which neuronal firing could be correlated with known concentrations of drug applied via the perfusing media (Pinnock, 1983). In the present study, the action of 3 different antipsychotics on the inhibitory response to dopamine was tested, to determine if the potencies of these agents in the nigral slice preparation correlated with data from biochemical binding and clinical investigations.

Methods

Male rats (150 g) were killed by a blow to the chest and their brain removed; 300 μ coronal sections of substantia nigra were cut using a vibrating sectioning system (Vibratome Oxford Instruments). The slices were maintained at 37°C in an oxygenated artificial cerebrospinal fluid (CSF), humidified, 95% O_2 plus CO_2 interface in a tissue chamber modified from a design by Haas *et al.* (1979). The flow rate through the chamber was 2 ml per min, the volume of the bath 0.3 ml. The artificial CSF was of the following composition (mM):- NaCl 124, KCL 2, KH_2PO_4 1.25, $MgSO_4$ 2, $CaCl_2$ 2, $NaHCO_3$ 25 and glucose 11.

Extracellular recordings were made by conventional techniques. Firing rates were displayed as ratemeter recordings with the number of spikes per 10, 15 or 20 s period on the vertical scale. Responses to dopamine were expressed as percentage inhibition of baseline firing rate before the addition of individual dopamine doses. Neurones visually identified as being in the zona compacta of the substantia nigra with the characteristics of dopamine cells (Guyenet & Aghajanian, 1978; Grace & Bunney, 1980) were selected to study the action of dopamine related

drugs. Most of these neurones responded to dopamine in the 1–100 μM range (Pinnock, 1983), those that failed to do so were discarded.

Drugs were made up in artificial CSF and the slice bathed in these solutions. Drugs were washed out by returning the slice to the original artificial CSF.

After a dose-response relationship for dopamine had been established on a single cell, a known concentration of the antagonist was perfused for five min. Then a new dose-response curve was obtained in the presence of the antagonist. The procedure was repeated until the cell was lost or the concentration of dopamine required to produce a response was in the mM range.

Each antagonist was tested at two or more different doses on a minimum of 3 cells. The concentration of dopamine required to produce 50% inhibition of firing was assessed by plotting dose-response curves using a linear regression program to obtain the best fit for the small number of points from individual neurones. In all cases the shifts of the dose-response curves in the presence of the antagonist appeared to be parallel. Schild plots for the results from each neurone were drawn. A Schild plot of the data from all the neurones treated with each antagonist was also calculated, this allowed data from neurones where only one dose of antagonist was tested to be included. The slope, intercepts and statistics were calculated using an analysis of covariance program on a computer (Snedecor & Cochran, 1937).

Results

Dopamine produced a dose-dependent depression of firing of neurones in the zona compacta of the substantia nigra. The sensitivity of neurones to dopamine varied over two orders of magnitude, with some cells responding to 0.1 μM dopamine while others would only respond to doses in excess of 30 μM. The great majority (17 out of 21) of neurones sampled had a threshold inhibitory response at 3 μM. Since the concentration range between threshold and 100% inhibition of firing of an individual neurone often covered less than two orders of magnitude, it is probable that 100% inhibition of firing does not represent a maximum response or saturation of receptors.

Low doses of all three antipsychotic drugs blocked the responses to dopamine, while even the highest doses of these agents had no effect on submaximal responses to γ-aminobutyric acid (GABA). The typical antagonism of dopamine responses by sulpiride, haloperidol and cis-flupenthixol is shown in Figure 1. The response to a given dose of dopamine remained constant over the period during which a given concentration of antagonist was applied, suggesting that the drugs were in equilibrium after 5 min (see Figure 1). Schild plots of the pooled data for (−)-sulpiride, haloperidol and cisflupenthixol are shown in Figure 3 and the pA_2 values given with the other data from the plots in Table 1.

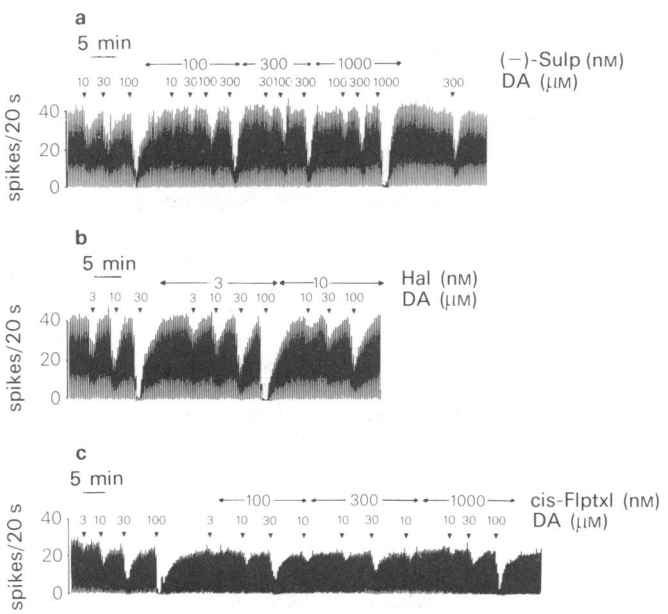

Figure 1 Ratemeter recordings from three different substantia nigra zona compacta neurones. Dopamine (DA) produced a dose-dependant depression of firing. (−)-Sulpiride ((−)-Sulp) (a), haloperidol (Hal) (b) and cis-flupenthixol (cis-Flptxl) (c) all antagonized the responses to dopamine. The antagonist actions could be overcome by increasing the doses of dopamine. Each application of dopamine lasted for one min.

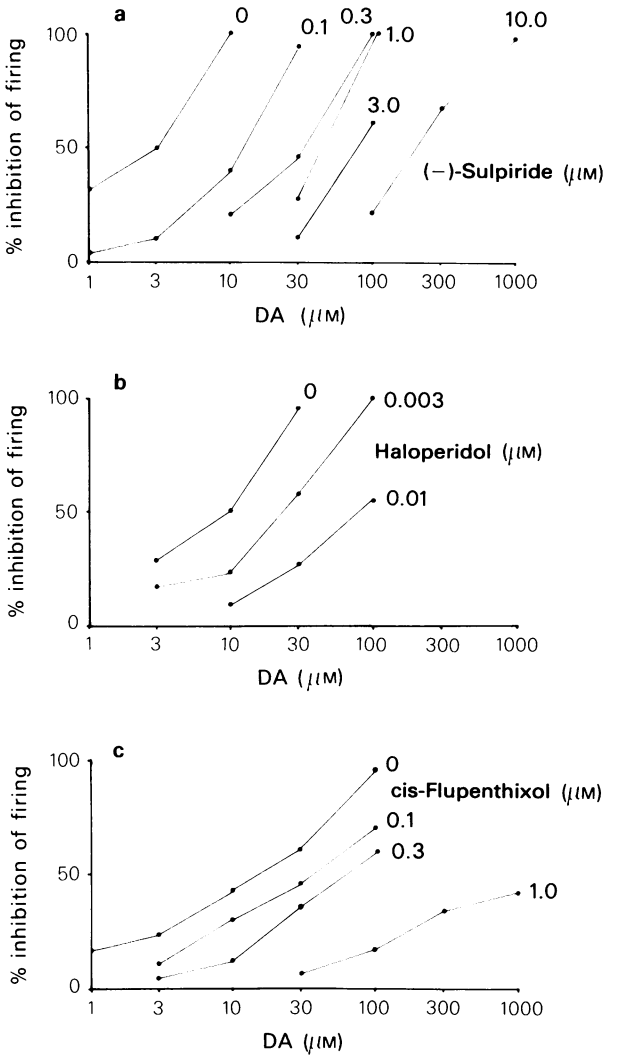

Figure 2 Dose-response curves from individual neurones, of the substantia nigra zona compacta, to dopamine (DA) in the presence of different concentrations of (a) (−)-sulpiride, (b) haloperidol and (c) cis-flupenthixol. It should be noted that 100% inhibition of firing may not represent maximum depression of the neurone.

Points on the graphs include those where initial shifts in the dose-response curves were small, producing negative log dose-ratio (DR) − 1 values. In spite of this Schild plots from individual neurones had correlation coefficients greater than 0.9 suggesting a good fit of lines to points. However, when the data from all the neurones on which an antagonist was tested were pooled the correlation coefficients were not so good (see Table 1).

Comparison of the regression lines showed that there was a significant difference between the intercepts of haloperidol and sulpiride ($P < 0.05$), haloperidol and cis-flupenthixol ($P < 0.05$), but not between sulpiride and cis-flupenthixol.

None of the Schild plots had slopes of unity; (−)-sulpiride was 0.7, cis-flupenthixol 1.8 and haloperidol 1.3. Since these are gross departures from the 0.95 for pA_2–pA_{10}, it suggests the antagonism was not competitive over the range of antagonist concentrations used to determine these values (see Discussion).

Extracellular recordings from single neurones in slices, followed by a minimum of 9 solution changes in order to obtain two points on a Schild plot, carried a high risk of failure as small movements of the tissue resulted in the loss of the neurone under study. While long recording times of up to 12 h were achieved it was impossible to predict when these would occur. Thus it was usual to obtain only two or three points on a dose-response curve under a given set of antagonist conditions. Hence, while the dose-response curves to dopamine in increasing concentrations of all three antagonists appeared to be displaced to the right in a parallel fashion in individual recordings (Figure 2), there were not enough data points to confirm this. Furthermore, the concentrations of dopamine needed to produce responses in the presence of high antagonist concentrations were in the mM range. At these levels non-specific and even osmotic actions are likely to complicate the results.

Of the three neuroleptics studied, only the antagonism by (−)-sulpiride could be reversed; a full response to the original dose of dopamine occurring after 2 h of washing. The effects of both haloperidol and cis-flupenthixol were not reversed during the time course of the experiment.

Table 1 The relative potencies of (−)-sulpiride, haloperidol and cis-flupenthixol at antagonizing the responses of neurones, in the zona compacta of the substantia nigra, to dopamine

	(−)-Sulpiride	*Haloperidol*	*cis-Flupenthixol*
		8.359	6.941
K_i (mol l^{-1})	2.985×10^{-8}	4.375×10^{-9}	1.148×10^{-7}
Slope of Schild plot	0.701	1.314	1.798
Correlation coefficient	0.936	0.739	0.803
n	11	11	9

Data are taken from the Schild plots shown in Figure 3.

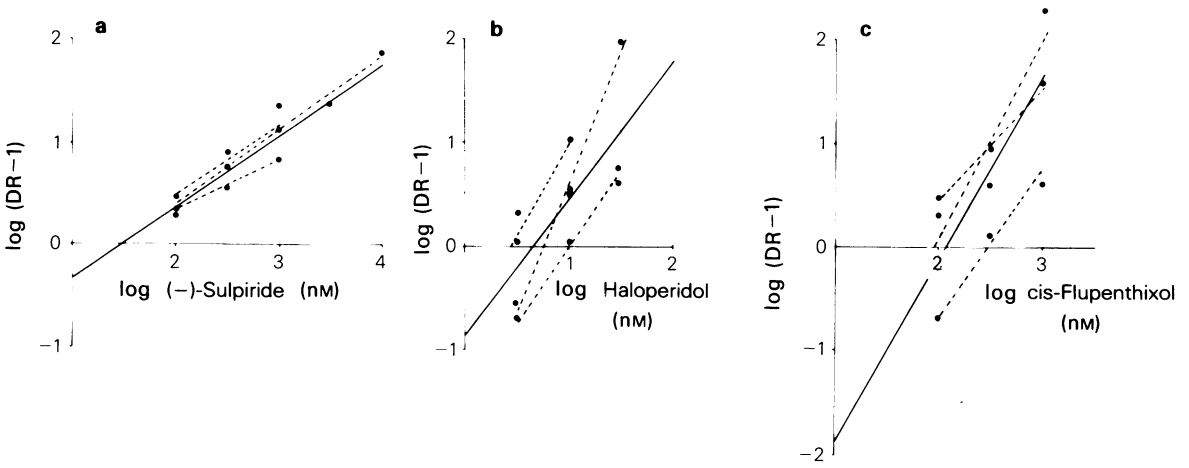

Figure 3 Schild plots showing the antagonism of dopamine by (a) (−)-sulpiride, (b) haloperidol and (c) cis-flupenthixol on the inhibitory responses of individual neurones of the substantia nigra zona compacta. The solid lines represent the best fit for all the data points. The dotted lines represent the best fit for points from individual neurones.

Discussion

The results of this study provide conclusive evidence that low doses of the antipsychotic drugs sulpiride, haloperidol and cis-flupenthixol antagonize dopamine responses at the neuronal level in the substantia nigra.

The (−) isomer of sulpiride has been shown to be more potent than the (+) isomer when iontophoretically applied to substantia nigra neurones (Woodruff & Pinnock, 1981). The potency of (−)-sulpiride (K_i 29 nmol l^{-1}) in the present study is similar to that seen in binding studies where IC_{50} values are in the 10 nmol l^{-1} range (Theodorou *et al.*, 1980; Freedman *et al.*, 1981a, b; Jenner & Marsden, 1981; Woodruff & Freedman, 1981). This is in contrast to other studies where IC_{50} values of 100–1000 nmol l^{-1} are common (see review by Seeman, 1980). However, the potency of sulpiride is limited in *in vivo* studies when administered peripherally because of its poor penetration of the blood brain barrier (Honda *et al.*, 1977; Woodruff & Andrews, 1979). This is clearly demonstrated when the results of the present study are compared with previous attempts, to block the depressant action of apomorphine on substantia nigra neurones, where very large doses of peripherally administered sulpiride are needed (Merev *et al.*, 1983). Thus sulpiride appears to be acting in a similar way to the classical neuroleptic drugs. The ease with which the action of sulpiride is reversed by washing compared with haloperidol and cis-flupenthixol, is possibly a reflection of its low oil/water partition coefficient (Norman & Drummond, 1979).

Haloperidol had a similar potency (K_i 4.4 nmol l^{-1}) to that predicted from *in vivo* binding (IC_{50} 1–10 nM) and clinical efficacy studies (Creese *et al.*, 1976; Seeman *et al.*, 1976; Seeman, 1980).

The thioxanthene, cis-flupenthixol, was less potent (K_i 150 nmol l^{-1}) than would be predicted from binding and clinical efficacy studies. The binding of cis-flupenthixol in striatum consists of two components which have been referred to as D_1 and D_2 sites. The affinity for both sites is similar, in the 1–10 nM (IC_{50}) range (Hytell, 1978; 1981). Furthermore cis-flupenthixol potently displaces sulpiride binding in the striatum (Freedman *et al.*, 1981c). Hence there is some discrepancy here between the present bioassay data and the results predicted from previous data.

Analysis of drug-receptor interactions using Schild regression is complicated if uptake inhibitors are absent. However, the use of such agents in the present study could not be considered, since spontaneous dopamine release might then inhibit neuronal activity thus removing the index used to measure dopamine responses. Since none of the Schild plots had slopes of unity, the antagonists do not appear to be acting competitively. However, in a comprehensive review Kenakin (1982) has discussed factors causing Schild slopes to deviate from unity. For example the antagonist may affect uptake or other transmitter function, or may not be in true equilibrium with the receptor. The latter is a possible explanation of the low potency of cis-flupenthixol, and it is possible that much longer exposure to individual doses of cis-flupenthixol would result in an apparent increase in potency.

In conclusion, the potency of (−)-sulpiride and

haloperidol but not cis-flupenthixol on dopamine receptors in the substantia nigra agrees well with that predicted from available data. However, the complexity of the bioassay system may distort the response occurring at the receptor level. In spite of the technical problems encountered and data not being amenable to complex statistical analysis, the CNS bioassay is a crucial link between biochemical response and clinical efficacy. This clearly justifies further studies of this kind.

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