in contractility with a minimum of (Na^+-K^+)ATPase inhibition.

In conclusion, the unusual properties of methyl-dtg-gluc with respect to the rapid onset and offset of the inotropic response is reflected by unusual kinetic features at the receptor level. The increased turnover rate of the complex between methyl-dtg-gluc and its receptor at a given level of equilibrium binding might have significance for the development of cardiac glycosides with improved therapeutic properties.

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On the mechanism of inhibition of dopamine receptors by fluphenazine*

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It is generally agreed that antipsychotic drugs exert their therapeutic and extrapyramidal effects by blocking dopamine receptors in the brain [1]. It is also often assumed that blockade is due to competitive inhibition of the association of dopamine to its receptors. Although antipsychotic drugs inhibit the specific binding of [^{3}H]dopamine ([^{3}H]DA) to membranes of rat [2–4] or calf [5] brain, the mechanism of this inhibition has not been determined. The finding that antipsychotic drugs inhibit the binding of [^{3}H]spiroperidol by a competitive mechanism [6, 7] cannot be used as evidence that the binding of dopamine to its receptors is also inhibited competitively by these drugs. For example, agonists and antagonists may bind to different sites [8] or to different subunits of a receptor complex [9]. Thus, although interactions within each class of compound may be competitive, interactions between agonists and antagonists may be more complex. Studies showing the multiphasic displacement of ^{3}H-agonists by antipsychotic drugs [2, 10] still do not address the mechanism whereby this displacement takes place.

In this study, we have investigated the inhibition of [^{3}H]DA binding by a potent phenothiazine neuroleptic, fluphenazine. The stereospecific and saturable binding of [^{3}H]dopamine was measured as we have previously described [11]. Briefly, caudate nuclei from adult male Sprague–Dawley rats were homogenized in 100 vol. of 50 mM Tris–HCl buffer, pH 7.0, containing 3.0 mM $CaCl_2$ and preincubated at 37° for 30 min. The homogenate was centrifuged at 20,000 *g* for 15 min at 4°, and the pellet was resuspended in 100 vol. of fresh buffer and recentrifuged. The pellet was washed in the same manner, and the final pellet was resuspended in 20 vol. of 50 mM Tris–HCl, pH 7.1, containing 5.0 mM tetrasodium EDTA, 10 mM $MgSO_4$ and 15 μM pargyline. Samples containing 0.25 mg tissue protein and 0.5 to 10.0 nM [^{3}H]DA (sp. act. 29 Ci/mmole, New England Nuclear Corp., Boston, MA), in a final volume of 0.1 ml buffer, were incubated for 30 min at 23° and rapidly filtered through GF/B filters under suction. The filters were washed with three portions of 5 ml buffer, and bound radioactivity was counted as previously described [11]. Total and nonspecific binding (in the presence of 10 μM *d*-butaclamol) was measured in triplicate. Typical values (± S.E.M.) of total binding were 2450 ± 34 cpm and of nonspecific binding 550 ± 20 cpm using 1 nM [^{3}H]DA or 7100 ± 125 and 2940 ± 60 cpm using 6 nM [^{3}H]DA.

The specific binding of [^{3}H]DA was saturable with a density (B_{max}) for 730 ± 30 fmoles/mg protein and a dissociation constant (K_d) of 1.41 ± 0.17 nM (means ± S.D. of six experiments). The B_{max} was higher that the value we reported previously [4, 11] because the present assay mix contained $MgSO_4$ which increases stereospecific [^{3}H]DA binding [2].

The addition of 5, 20 or 100 nM fluphenazine–HCl to the binding assay lowered the B_{max} progressively without changing the K_d (Fig. 1A). Higher concentrations of fluphenazine (500 or 1000 nM) induced significant increases in the K_d. The results of these and other experiments with additional fluphenazine concentrations are summarized in Fig. 1B. It is clear that concentrations of fluphenazine between 5 and 100 nM inhibited [^{3}H]DA binding by lowering the B_{max}, whereas higher drug concentrations decreased receptor affinity as well.

These results suggested that fluphenazine inhibited [^{3}H]DA binding either noncompetitively or irreversibly. The latter can be evaluated by measuring binding to several receptor concentrations in the presence of a constant concentration of inhibitor. An irreversible inhibitor removes a constant amount of receptors from the binding reaction, shifting the intercept rather than the slope of the protein-binding function (Ref. 12, p. 128). In contrast,

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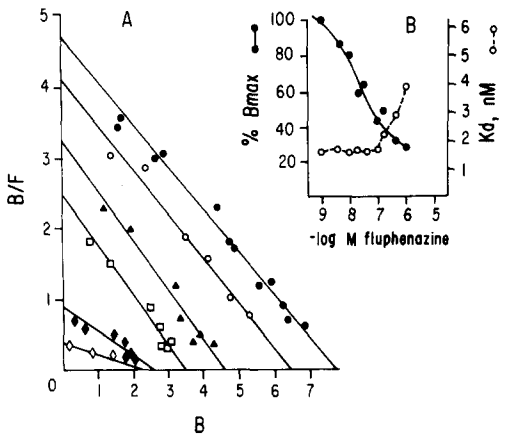

Fig. 1. Inhibition of saturable [^{3}H]DA binding by fluphenazine. Panel A: Scatchard plots of saturation isotherms obtained with 0.5 to 10.0 nM [^{3}H]DA and increasing concentrations of fluphenazine. Key: (●) control (results from two independent experiments were combined); (○) 5 nM fluphenazine; (▲) 20 nM; (□) 100 nM; (◆) 500 nM; and (◇) 1000 nM; (B) stereospecific binding in fmoles/mg protein; and (F) free [^{3}H]DA in nM. Each point is the mean of triplicate determinations. The lines were fitted by a linear regression program. Panel B: Results from experiments shown in panel A and from additional saturation experiments, performed under identical conditions using 1, 10, 30 or 200 nM fluphenazine. The K_d values were the slopes and the B_{max} values were the x-axis intercepts of Scatchard plots obtained as shown in panel A. The standard deviation of these parameters was less than 10%, calculated with a linear regression program.

fluphenazine decreased the slope but did not shift the x-intercept to the right, behaving as a true noncompetitive, and not an irreversible, inhibitor (Fig. 2).

It has been suggested that the decrease in the B_{max} of [^{3}H]spiroperidol induced by agonists [7] is due to the saturation of a subset of sites with high affinity for agonists rather than to true noncompetitive inhibition. Insofar as [^{3}H]DA binding also involves sites with high- and low-antagonist affinities [2], an analogous phenomenon may occur with the antagonist-induced displacement of this radioligand. Accordingly, the displacement of specific [^{3}H]DA binding by spiroperidol was biphasic, whereas the fluphenazine-induced displacement was monophasic (Fig. 3). If fluphenazine saturated the sites with high-antagonist affinity, then a maximal 30–35% decrease in the B_{max} would have been observed. Moreover, pseudononcompetitive inhibition would occur only with inhibitor concentrations which saturate the sites with high-antagonist affinity. Lower fluphenazine concentrations would yield biphasic Scatchard plots of [^{3}H]DA binding, as the K_d at the sites with high-antagonist affinity increased due to a competitive interaction with fluphenazine and the remaining sites were unaffected. Higher drug concentrations would result in apparent mixed inhibition as the saturation of the sites with high-antagonist affinity would decrease the B_{max}, and competitive inhibition of the remaining sites would increase the K_d. However, the predictions of the pseudononcompetitive model of inhibition were not experimentally confirmed. The inhibition of [^{3}H]DA binding was noncompetitive over a wide range of fluphenazine concentrations (Fig. 1), and the B_{max} was suppressed by 60% before increases in the K_d were observed. Moreover, concentrations of fluphenazine too low to saturate the high-antagonist affinity site still resulted in noncompetitive inhibition and not in biphasic Scatchard plots.

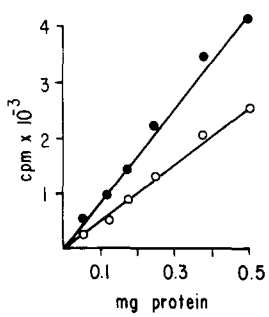

Fig. 2. Effect of caudate nucleus protein concentration on the specific binding of [^{3}H]DA in the presence or absence of fluphenazine. The binding of a saturating concentration of [^{3}H]DA (7.0 nM) was measured with the indicated protein concentrations as described in the text. Units of ordinate; stereospecific cpm in thousands. Key: (●) control; and (○) 100 nM fluphenazine. Each point is the mean of quadruplicate determinations, the standard deviation of which was less than 10%. The slopes of the two lines are significantly different ($P < 0.01$), determined by an appropriate t-test [13].

Rothman and Westfall [14] have reported that the binding of [^{3}H]leucine enkephalin to rat brain membranes was noncompetitively inhibited by morphine, and they attributed this to an allosteric mechanism. Similarly to what we describe with fluphenazine, the effects of morphine were concentration-dependent and reversible [14]. The concept of reversible noncompetitive inhibition of binding presents great theoretical difficulties. There is no analogy with noncompetitive enzyme inhibition, because a noncompetitive enzyme inhibitor has no effect on substrate binding (Ref. 12, p. 125) but on product formation. The reversible binding of an agonist (D) and an antagonist (I) to different sites on a receptor (R) is analogous to partial competitive inhibition (Ref. 12, p. 161 ff.) and leads to an equation of the form

$$\frac{[RD] + [RDI]}{[R_t]} = \frac{[D]}{K_d \dfrac{(1 + [I]/K_i)}{(1 + [I]/\alpha K_i)} + [D]}$$

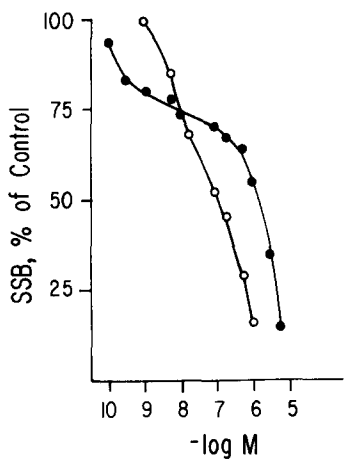

Fig. 3. Inhibition of specific [^{3}H]DA binding by fluphenazine or spiroperidol. The binding of 3.0 nM [^{3}H]DA to rat caudate nucleus membranes (0.25 mg protein) was measured as described in the test. Total binding was 4480 ± 133 cpm, 950 ± 102 of which was nonspecific (displaced by 10 μM d-butaclamol). SSB: stereospecific binding. Key: (●) spiroperidol; and (○) fluphenazine.

(cf. Ref. 12, equation IV-2). This gives a linear Scatchard plot with no decrease in B_{max}. We are left, therefore, with no obvious model for reversible noncompetitive binding inhibition.

If we ignore this theoretical problem, and simply assume that B_{max} has two components, B_1 and B_2, the first being somehow noncompetitively inhibited by fluphenazine (5–100 nM), we have

$$B_{max} = B_1/(1 + [I]/K_i) + B_2$$

where $[I]$ is the concentration of fluphenazine and K_i its inhibitor constant. Fitting this to the data on B_{max} (Fig. 1) by using a least-squares curve fitting computer program, we obtain $B_1 = 64\%$ of B_{max} and $K_i = 18$ nM. This value of the fluphenazine-receptor dissociation constant agrees with reported estimates of the IC_{50} of the fluphenazine-induced inhibition of [^{3}H]spiroperidol binding to D-2 receptors [1]. It is, therefore, possible that fluphenazine regulates the high-affinity binding site of [^{3}H]DA allosterically through an interaction with the site labeled by ^{3}H neuroleptics.

In summary, our results suggest strongly that fluphenazine inhibits the major portion of the specific binding of [^{3}H]DA to its high-affinity receptors in rat striatum via a noncompetitive mechanism. Because there is no evident theoretical model for reversible noncompetitive binding inhibition, this interpretation must be approached with caution. Insofar as a similar mechanism was described for the morphine-induced displacement of [^{3}H]leucine enkephalin [14], the noncompetitive inhibition of neurotransmitter-receptor binding by antagonists may merit further investigation.

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Effect of ACTH on ornithine decarboxylase activity of adrenal medulla and cortex

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ACTH administration stimulates the induction of ornithine decarboxylase (ODC, EC 4.1.1.17) activity in the adrenal gland of experimental animals [1–3]. Thus far, there are results only for whole adrenal glands, and the effect of the hormone on the separate tissues making up the adrenal has not been determined. Our previous studies of the mechanisms of induction of adrenomedullary and -cortical ODC have indicated that for the chromaffin tissue the increase of activity caused by various stressors and drugs is mediated largely by neural mechanisms involving intact splanchnic innervation of the gland [4, 5] whereas the increase in adrenal cortex under the same conditions is mediated to an important extent by a hormonal component (*ibidem*). It is clearly important to establish the relation of induction by ACTH to the increases in ODC engendered by stressing animals. The effect of ACTH on ODC activity of the two separate tissues has now been studied.

Hypophysectomized male Sprague–Dawley rats, weighing about 200 g, were purchased from Canadian Breeding Farms and Laboratories Ltd., St. Constant, Quebec. They were kept in the animal room in individual wire cages under a light–dark cycle of 12:12 hr, with tap water and Purina Checkers *ad lib*. Five days after hypophysectomy, the rats received one subcutaneous injection of synthetic ACTH (10 I.U., equivalent to 0.1 mg/rat). Four to six animals were killed every 2 hr up to 12 hr, along with two additional groups at 18 hr and 24 hr after receiving ACTH. Adrenal medulla and cortex were separated at 4° by dissection under a magnifying lamp as previously described [4, 6]. The tissue corresponding to two medullae or two cortices was pooled and homogenized in 200 μl of Na^+–K^+–phosphate buffer, 0.05 M, pH 6.8, with a motor-driven Teflon homogenizer. The homogenate was centrifuged at 20,000 *g* for 20 min, and the supernatant fraction was taken for assay of ODC activity [4].

Sham-operated rats were used as controls in some experiments. They received 0.1 ml of a solution of 1% methylcellulose, instead of the ACTH solution. Otherwise their treatment was the same as above.

The results with hypophysectomized rats are shown in Fig. 1. Adrenomedullary ODC did not respond to ACTH administration in the time-course studied. On the other