THE ACTIONS OF FLUPENTHIXOL UPON 5-HYDROXYTRYPTAMINE-INDUCED AGGREGATION AND THE UPTAKE OF 5-HYDROXYTRYPTAMINE AND DOPAMINE BY HUMAN BLOOD PLATELETS

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The effects of the α- and β-isomers of flupenthixol on 5-hydroxytryptamine (5-HT)-induced platelet aggregation and on 5-HT and dopamine uptake were investigated. α-Flupenthixol was 185 times more potent than the β-isomer as an inhibitor of platelet aggregation. In contrast both isomers were equipotent as inhibitors of uptake of 5-HT and dopamine. The data suggest that 5-HT-induced aggregation and uptake are separate processes.

Introduction We are currently attempting to relate changes in the therapeutic efficacy of neuroleptic drugs used for the treatment of psychosis to changes in blood platelet aggregation responses. The basis for these experiments is that while Mills & Roberts (1967) and Boullin, Grahame-Smith, Grimes & Woods (1975a) found that chlorpromazine *in vitro* inhibited platelet aggregation responses to 5-hydroxytryptamine (5-HT), we subsequently observed enhanced aggregation responses to 5-HT in schizophrenics chronically treated with chlorpromazine (Boullin, Woods, Grimes, Grahame-Smith, Wiles, Gelder & Kolakowska, 1975b).

As the thioxanthine derivative flupenthixol, which is structurally related to the phenothiazines, is currently in use for the treatment of psychosis (Reiter, 1969), we thought it would be of interest to study the aggregation responses of patients treated with flupenthixol. However, recent work suggested to us that some preliminary experiments upon the effects of flupenthixol isomers *in vitro* should be made.

Flupenthixol shows geometrical isomerism existing as *cis* (α) and *trans* (β) forms. The α-form shows the greatest biological activity in the following experimental situations relating to central dopaminergic mechanisms: inhibition of dopamine-induced stimulation of adenylate cyclase of rat striatum (Miller, Horn & Iversen, 1974); inhibition of turning behaviour in rats induced by methamphetamine or apomorphine following the production of unilateral lesions of the substantia nigra with 6-hydroxydopamine (Kelly & Miller, 1975).

Additionally the α-isomer of flupenthixol is more potent than the β-isomer in a large number of behavioural tests in various species (Møller, Nielsen, Pedersen, Nymark, Franck, Boeck, Fjalland & Christensen, 1973).

In view of these results and the possible involvement of central dopaminergic mechanisms we have studied the effects of α- and β-flupenthixol upon platelet aggregation and upon 5-HT and dopamine uptake by platelets.

Methods Citrated platelet rich plasma (PRP) was prepared as described by Boullin, Green & Price (1972). 5-HT-induced aggregation was measured as described in previous papers (Boullin *et al.*, 1975a; Boullin *et al.*, 1975b).

The α- and β-isomers of flupenthixol were added to 1 ml samples of PRP at 37° C, 3 min before addition of 20 μM 5-HT to induce aggregation. The inhibitory potency of flupenthixol isomers was expressed as percentage inhibition determined by the total change in optical density of PRP produced by 5-HT in the presence of various concentrations of flupenthixol compared with the aggregation responses produced by 5-HT alone (Boullin *et al.*, 1975a).

5-HT and dopamine uptake into platelets was measured as described previously by Boullin & O'Brien (1970) using concentrations of 0.1 to 10 nmol/ml.

Results Both α- and β-isomers of flupenthixol inhibited 5-HT-induced aggregation and also 5-HT and dopamine uptake (Figure 1). However, there were differences in the inhibitory potencies of the two isomers in regard to these parameters. For example the ED_{50} for inhibition of 5-HT-induced aggregation was 0.07 nmol/ml for α-flupenthixol and 13.0 nmol/ml for the β-isomer. On the other hand, there was no difference in potency between the isomers in regard to inhibition of 5-HT or dopamine uptake. The ED_{50} for inhibition of 5-HT

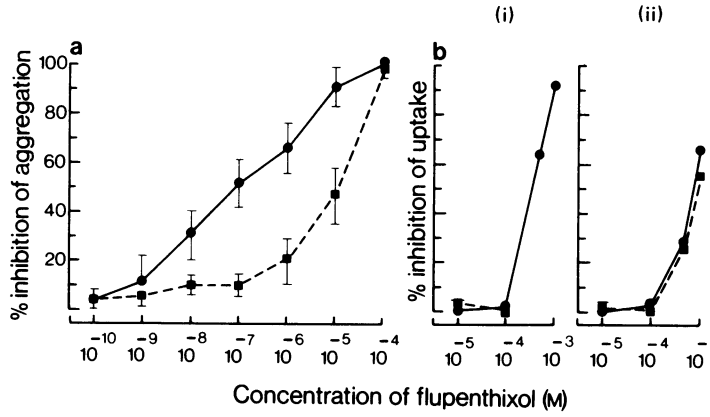

Figure 1 (a) Inhibition of platelet aggregation by α-flupenthixol (•), and β-flupenthixol (▪). Aggregation was induced by 20 nmol/ml 5-hydroxytryptamine (5-HT). The values shown are the mean of six experiments. Vertical lines show s.e. mean. (b) Inhibition of the uptake of 10^{-6} M 5-HT (i) and 10^{-6} M dopamine (ii) by α- (•) and β (▪) flupenthixol. The inhibition of 5-HT uptake by 10^{-4} M and 10^{-3} M flupenthixol was identical for both isomers and only one line is drawn (i). Each value is the mean of two determinations. Similar results were obtained using substrate concentrations of 10^{-5} M and 10^{-7} M. Note that there is a difference in potency between the two isomers only in regard to inhibition of aggregation.

uptake was 50 nmol/ml and 90 nmol/ml for dopamine uptake.

Discussion The data show that flupenthixol is an inhibitor *in vitro* of 5-HT-induced platelet aggregation. The ID_{50} for inhibition by the α-isomer is 0.07 nmol/ml which is comparable to the potency of the most active chlorpromazine metabolite (NOR_1 chlorpromazine) investigated in our earlier experiments (Boullin *et al.*, 1975a). Also of interest in regard to our clinical investigations (Boullin *et al.*, 1975b) is the fact that we have observed enhanced aggregation responses to 5-HT in a small number of patients receiving αβ-flupenthixol which is the form of the drug in therapeutic use (Present authors' unpublished observations). Thus there seems little doubt that flupenthixol is a suitable compound for investigating changes in the relationship between platelet aggregation responses and clinical effect in patients suffering from psychoses.

Regarding the pharmacological activity of flupenthixol isomers, our results confirm earlier reports (cited in the introductory section) that α-flupenthixol is more potent than the β-isomer in various pharmacological test systems.

It is of interest that the difference in potency between the two isomers of flupenthixol on platelet responses only applies to the physiological response of the cells to 5-HT, namely aggregation, and not to changes in 5-HT or dopamine uptake. These differences support the view that amine uptake and aggregation processes are dissociated in platelets. Bygdeman & Johnson (1971) first reported a dissociation between noradrenaline-induced aggregation and uptake, while Born, Juengjaroen & Michal (1972) reached a similar conclusion with 5-HT. Our results agree with the viewpoint of the earlier workers, suggesting that the mechanisms involved in 5-HT-induced platelet aggregation are quite different from those involved in 5-HT uptake. Furthermore, our results indicate that the differences in potency between the two isomers of flupenthixol are not confined to dopaminergic systems (Kelly & Miller, 1975) but also extend to the 5-HT-mediated aggregation response.

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