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RELATIONSHIP BETWEEN THE ABILITY OF SOME NEUROLEPTICS TO ENHANCE STRIATAL [^{3}H]DOPAMINE RELEASE AND THEIR LIPOPHILICITY

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Abstract—The ability of micromolar concentrations (100 μM) of some neuroleptics to enhance release of [^{3}H]dopamine from rat striatum was studied. All the neuroleptics tested potentiated [^{3}H]dopamine release, the most potent being chlorpromazine, prochlorperazine and fluphenazine, and the least potent being sulpiride and metoclopramide. Apomorphine did not reverse the effects of fluphenazine and the stereo-isomers of flupenthixol were almost equipotent. These results indicated a direct, non-receptor, mediated mechanism. The lipophilicity of the neuroleptics, determined by reversed-phase TLC, showed a good correlation with their ability to enhance dopamine release. It is concluded that the lipophilicity of these drugs is partly responsible for the enhanced release of dopamine and may be clinically important following chronic administration of neuroleptics.

Neuroleptic drugs are thought to modulate catecholamine function in the central nervous system by blocking dopamine receptors and this interaction appears to be a prerequisite for neuroleptic activity (for a review see Seeman [1]). Thus, there is a good correlation between the antipsychotic potencies of butyrophenones and phenothiazines and their affinities in competing for *in vitro* binding of [^{3}H]haloperidol to dopamine receptors in calf and rat striatum [2,3]. Atypical neuroleptics, such as benzamide derivatives, have also been shown to be selective neuroleptic agents [4]. In general, the concentrations of neuroleptics required to inhibit [^{3}H]haloperidol and spiroperidol binding to rat or calf striatum are in the nmolar range.

However, at relatively high (μmolar) concentrations, neuroleptics have been reported to alter neurotransmitter release from rat striatum via non-receptor mechanisms [5,6]. Since neuroleptics are potent surface-active agents (e.g. ref. [28]), the aim of the present study was to determine whether any relationship existed between the lipophilic nature of phenothiazines and benzamide derivatives and their ability to enhance striatal dopamine release.

MATERIALS AND METHODS

Chemicals and drugs. [7,8-^{3}H]Dopamine (43 Ci/mmole) was obtained from Amersham International. NCS tissue solubilizer was obtained from Amersham/Searle and 'Fisofluor 1' from Fisons Scientific Apparatus. Methohexitone Na ('Brietal Na') and Apomorphine-HCl were obtained from Eli Lilly and Sigma Chemicals, respectively. The following drugs were gifts: trifluoperazine (Smith, Kline & French); thioridazine-HCl (Sandoz Products); pericyazine, prochlorperazine dimaleate and chlorpromazine-HCl (May & Baker); Metoclopramide-HCl (Beechams); *cis*(*Z*)- and *trans*(*E*)-flupenthixol-diHCl (H. Lundbeck); sulpiride (Delagrange). The drugs were dissolved in a physiological medium (for composition see below) or dimethyl suphoxide and then diluted in physiological medium. Dimethyl sulphoxide at the concentration used (0.5% v/v) had no effect on the release of dopamine itself.

Preparation and superfusion of striatal tissue. Male Wistar rats were decapitated and the dissected striatum (45–55 mg) chopped into miniprisms (0.1 × 0.1 × approximately 1.0 mm) by a McIlwain tissue chopper. The tissue was re-suspended (5 mg tissue/ml) in ice-cold physiological medium (133 mM NaCl, 5 mM KCl, 2.7 mM $CaCl_2 \cdot 2H_2O$, 1.2 mM $MgSO_4 \cdot 7H_2O$, 10 mM Tris–HCl, 10 mM glucose and fresh 1 mM L-ascorbic acid) and samples (1 ml) diluted with 9 ml physiological medium and pre-incubated at 37° for 15 min. [^{3}H]Dopamine (1 μCi; 0.03 μM) was added to the tissue suspensions and incubation, with shaking, was continued for a further 10 min at 37°.

The tissue suspensions were poured onto Whatman GF/B filters in superfusion chambers maintained at 37° as described by Raiteri *et al.* [7]. After washing with a further 10 ml of physiological medium, the tissue beds were superfused with medium at a rate of 1 ml/min. Under these conditions, re-uptake of released neurotransmitter was prevented by virtue of the continuous perfusion [8].

Fractions of the superfusate were collected after a steady basal efflux rate was obtained (normally after 4–5 min) and when the medium in each chamber was almost exhausted, a submaximal depolarizing

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concentration of K^+ was added. Superfusate fractions were collected for a further 9 min after which excess medium was drawn off. The drugs under study were either added with the depolarizing concentration of K^+ or incubated with the striatal tissue as described in the Results.

Radioactivity in the fractions was counted by liquid scintillation spectrophotometry in 5 ml 'Fisofluor 1' (containing 10% ethoxyethanol v/v). The tissues left on the filters were solubilized with 0.5 ml NCS tissue solubilizer and counted in 9.5 ml 'Fisofluor 1'. The efficiency of counting was in the range 30–35% and results were corrected to dpm using the external standard procedure. The radioactivity present in each fraction was calculated as a percentage of the total radioactivity recovered from that chamber (i.e. the radioactivity in all the fractions collected + tissue + filter).

Reversed-phase TLC. To determine the lipophilicity of the drugs under study, a reversed-phase TLC method was used as described by Hulshoff and Perrin [9]. The technique uses an adsorbent (e.g. cellulose) as support for a non-aqueous stationary phase and can yield partition coefficients for compounds between organic solvents, e.g. *n*-octanol and water. However, in this study the absolute partition coefficients were not calculated, but simply the R_f values (for the relationship between the R_f value in partition chromatography and the partition coefficient P see Ref. 9). This approach provided a simple but reliable evaluation of the lipophilicity, because all the drugs tested were spotted on the same plate and the relative positions calculated.

Plastic plates (20 × 20 cm), covered with a 0.25 mm layer of cellulose (Schleicher & Schüll), were impregnated with *n*-octanol (the non-aqueous stationary phase) by developing the plates in ether–*n*-octanol (95:5, v/v). The ether was allowed to evaporate at room temerature for 8 hr. Samples (5 μl) of drugs (5–10 μg) were spotted and the plates developed in phosphate buffer, pH 7.4 (the mobile phase), at a temperature of 25–27°. In all cases acetone, 5% (v/v), was added to the aqueous phase to enable the more lipophobic drugs to move from the origin [9]. After development, the drugs were detected either by visualization under u.v. or by spraying with the following reagents: potassium iodoplatinate reagent [10] or conc. H_2SO_4–H_2O–95% ethanol (1:1:8, by volume). The mean R_f value of each neuroleptic was calculated from plates developed on three separate runs.

Statistical treatment of results. Results are expressed as the means ± S.E. with the number of replicate experiments given in parentheses (below). Comparison between the effects of drugs and controls were analysed by an independent Student's *t*-test. Linear regression analysis was used to analyse the effects and lipophilicity of drugs and the correlation coefficient determined.

RESULTS

K^+-evoked release of radiolabel from striatum

Preliminary experiments showed that the concentration of K^+ required to evoke release of radiolabel was dose-dependent. Thus, at concentrations of 15, 25 and 40 mM K^+, the percentage increase over corresponding basal efflux was 22.2 ± 4.5 (6), 41.5 ± 5.5 (4) and 71.7 ± 14.4% (3), respectively. Addition of further 5 mM K^+ medium had no effect on basal efflux. In subsequent experiments, a submaximal concentration of 15 or 25 mM K^+ was used, so that either facilitation or inhibition of release could be observed.

The identity of the radiolabel in the superfusate was determined by TLC on Linear K silica gel (Whatman) in *n*-butanol–acetic acid–H_2O (4:1:1, by volume). A typical experiment showed that 74 and 87% of the ^3H released during the basal efflux and K^+-evoked period, respectively, co-migrated with authentic dopamine. Replacement of Ca^{2+} with Mg^{2+} in the medium, resulted in a reduction of 60% in the K^+ evoked release.

Effect of fluphenazine-HCl *on* [^{3}H]*dopamine release*

In these experiments, tissue was incubated for 10 min with [^{3}H]dopamine, as described in the Materials and Methods, followed by a further 5 min incubation period in the presence of fluphenazine (100 μM) before superfusing. This procedure enabled effects on basal efflux to be observed. Experiments where fluphenazine (100 μM) was present in the pre-incubation period (15 min) and the incubation with [^{3}H]dopamine (10 min) showed the total radioactivity recovered was identical compared to that recovered using the method above, indicating that fluphenazine did not markedly inhibit striatal uptake of [^{3}H]dopamine. Figure 1 shows that concentrations of 1–10 μM fluphenazine do not significantly alter the basal efflux of K^+-evoked release. However, at a concentration of 100 μM, the basal

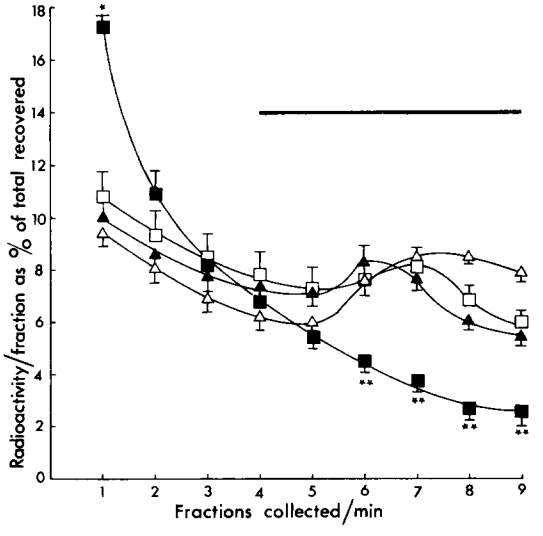

Fig. 1. Basal efflux and K^+-evoked release of [^{3}H]dopamine with no drug present (▲) and in the presence of 100 μM (■), 10 μM (△) and 1 μM (□) fluphenazine. The solid bar represents the presence of high (15 mM) K^+ medium. Each point is the mean ± S.E. of 3–7 independent experiments. Comparisons between the control and drug release: *$P < 0.005$; **$P < 0.001$.

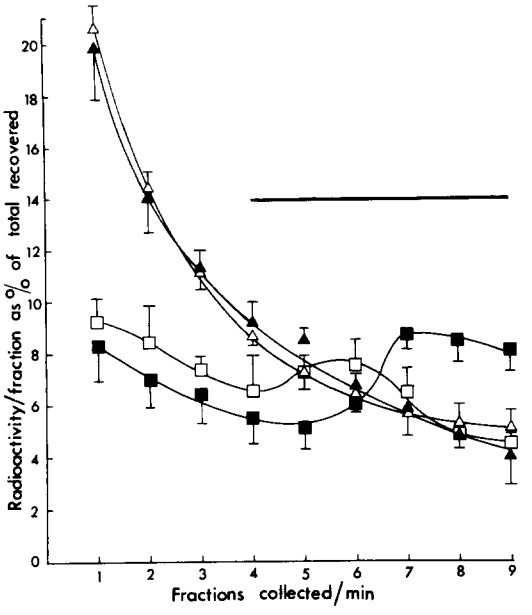

Fig. 2. Basal efflux and K^+-evoked release of [^{3}H]dopamine with no drug present (□) and in the presence of 100 μM apomorphine (■), 100 μM fluphenazine (▲) and 100 μM apomorphine + 100 μM fluphenazine (△). The solid bar represents the presence of high (15 mM) K^+ medium. Each point is the mean ± S.E. of three or four independent experiments.

efflux is initially elevated ($P < 0.005$) compared to the control level and then rapidly decreases, despite the addition of 15 mM K^+ medium. The inhibition of K^+-evoked release in fractions 6–9 is significant ($P < 0.001$).

The dopamine receptor agonist, apormophine, could not reverse the enhanced basal efflux and inhibition of K^+-evoked release caused by fluphenazine (Fig. 2). Thus, the profile of dopamine release in the presence of apomorphine (100 μM) and fluphenazine (100 μM) was identical to that caused by fluphenazine alone. Note that apomorphine (100 μM) alone had no significant effect on basal efflux but did appear to potentiate K^+-evoked release. This observation was not further investigated.

Effects of phenothiazines and atypical neuroleptics on K^+-evoked release

In these experiments, the drugs were not incubated with tissue suspension (therefore, their effects on basal efflux could not be determined), but were present in the high-K^+ (25 mM) medium. The results are expressed as a peak K^+-evoked release, which was determined by the highest value of the percentage released over the basal efflux, in the 4–9 min K^+ exposure. The results in Table 1 show that all the phenothiazines, *cis*- and *trans*-flupenthixol and, to a lesser extent, the benzamide derivatives, enhanced the K^+-evoked release of [^{3}H]dopamine compared to that obtained with no drug added. The increases in K^+-evoked release caused by all the neuroleptics, except metoclopramide, were statistically significant (Table 1).

Drug concentrations of 100 μM were chosen, thereby ensuring that effects on dopamine release were not mediated through dopamine receptors. In this respect, *cis*-flupenthixol only caused a 1.8-fold increase in K^+-evoked release compared to *trans*-flupenthixol.

Addition of a barbiturate, methohexitone sodium (100 μM), had no significant effect on the K^+-evoked release of dopamine (Table 1).

Table 1. Apparent lipophilicity of neuroleptics and their effects on the K^+-evoked release of [^{3}H]dopamine

	R_f	Peak K^+-evoked release (as % increase over basal efflux)
Chlorpromazine	0.43 ± 0.01	159 ± 36***
Prochlorperazine	0.22 ± 0.04	158 ± 55*
Fluphenazine	0.44 ± 0.04	159 ± 31***
cis-Flupenthixol	0.43 ± 0.03	130 ± 26**
Thioridazine	0.44 ± 0.01	106 ± 10*
Pericyazine	0.57 ± 0.03	97 ± 15*
Trifluoperazine	0.51 ± 0.01	96 ± 12*
trans-Flupenthixol	0.52 ± 0.03	91 ± 17*
Sulpiride	0.90 ± 0.01	89 ± 8*
Metoclopramide	0.85 ± 0.02	85 ± 17
Methohexitone Na	—	27 ± 5

The R_f values of neuroleptics were determined by reversed-phase TLC in *n*-octanol–H_2O as described in the Materials and Methods (means ± S.E.; $n = 3$). The peak K^+-evoked release caused by 25 mM K^+ medium with no drug added was 42 ± 10% ($n = 7$). Results are means ± S.E. of three [neuroleptics (100 μM) + 25 mM K^+-medium] independent experiments. A significant enhanced release was shown by the neuroleptics compared to the release with no drug added.

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.005$.

Reversed-phase TLC of neuroleptics

Neuroleptics were chromatographed as described in the Materials and Methods and the R_f values calculated. Table 1 shows the most lipophilic (the smaller R_f value) phenothiazine tested was prochlorperazine whilst the least lipophilic drugs (the greater R_f value) were the benzamide derivatives, sulpiride and metoclopramide. R_f values for the other phenothiazines and a thioxanthene, *cis*- and *trans*-flupenthixol, ranged between these two extremes. In comparison, the theoretical Hansch $\Sigma\pi$ values ($\Sigma\pi = \log P$ if P is the true partition coefficient) for some of the neuroleptics were calculated according to Tute [11]. For example, the $\Sigma\pi$ values for prochlorperazine and chlorpromazine were 5.53 and 5.35, respectively, and for pericyazine, sulpiride and metoclopramide, 3.91, 2.06 and 0.98, respectively. Therefore, the calculated $\Sigma\pi$ values support the experimental R_f values in determining the relative lipophilicity of the drugs tested.

Relationship between lipophilicity of neuroleptics and enhancement of K^+-evoked release of dopamine

There is a good correlation (Fig. 3) between the lipophilic nature of six phenothiazines, a thioxanthene and two benzamide derivatives and their ability at μmolar concentrations to enhance the K^+-evoked release of [^{3}H]dopamine from striatal tissue, i.e. the more lipophilic the neuroleptic the greater the increase in K^+-evoked release. The correlation is statistically significant ($r = 0.727$; $P < 0.02$).

DISCUSSION

The results demonstrate that fluphenazine, at a concentration of 100 μM increases the basal efflux and inhibits the K^+-evoked release of [^{3}H]dopamine from striatal miniprisms. This effect could not be reversed by equimolar concentrations of the dopamine receptor agonist, apomorphine. Mitchell [5], using the miniprism preparation, also showed neuroleptics to cause concentration-dependent increases in basal [^{3}H]dopamine efflux and inhibiton of stimulus-evoked release. The IC_{50}s for typical neuroleptics were in the range 1–4 μM, whilst benzamide derivatives had IC_{50}s greater than 400 μM. It was suggested that the blockade of evoked release which paralleled its enhancement was probably caused by the elevated baseline efflux. The initial enhanced release caused by 100 μM fluphenazine probably causes a large depletion of [^{3}H]dopamine stores and, if the releasing pool of dopamine was greatly depleted, then the stimulus-evoked response would be difficult to detect. This suggestion is supported by preliminary experiments which show that the counts, present in the tissue 5 min after incubation with 100 μM fluphenazine (33,252 dpm/5 mg tissue), were depleted approximately 64% compared to the control condition with no drug present (92,547 dpm/5 mg tissue) (M. W. Goosey, unpublished result). The *trans*-isomer of flupenthixol (a poor antagonist of dopamine receptors) produced the same alterations of dopamine release as the active *cis*-isomer.

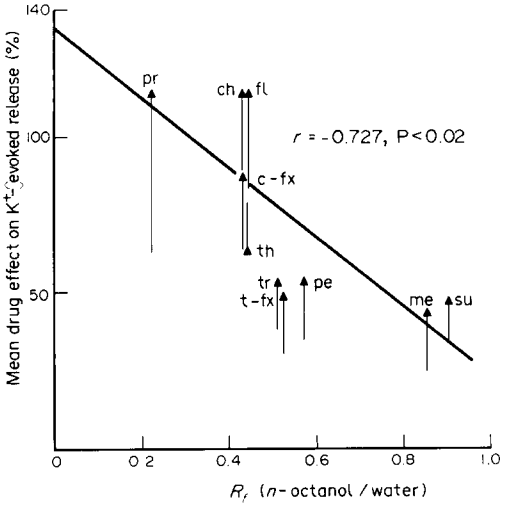

Fig. 3. The enhanced K^+-evoked release of [^{3}H]dopamine by six phenothiazines, one thioxanthene and two benzamide derivatives correlated with the R_f values obtained by reversed-phase TLC in *n*-octanol–water. Linear regression analysis yielded a correlation coefficient of −0.727, which is significant at the $P < 0.02$ level. Error bars represent S.E. Abbreviations: pr, prochlorperazine; ch, chlorpromazine; fl, fluphenazine; c-fx, *cis*-flupenthixol; th, thioridazine; tr, trifluoperazine; t-fx, *trans*-flupenthixol; pe, pericyazine; me, metoclopramide; su, sulpiride.

When the neuroleptics were present only in the K^+-depolarization medium, an enhanced release of [^{3}H]dopamine was observed. Thus, by either adding the neuroleptics before or with the depolarizing concentration of K^+-medium, an increase in the basal efflux or K^+-evoked release, respectively, occurs. The enhanced release of dopamine is most likely the result of non-specific membrane actions, since apomorphine did not reverse this effect and no stereoselective actions were found for the stereoisomers of fluphenthixol. The stereoselective actions of neuroleptic isomer pairs, such as (+)- and (−)-butaclamol and *cis*- and *trans*-flupenthixol, are required to identify truly specific interactions with dopamine receptors (e.g. Seeman and Lee [12]). Therefore, these results using the miniprism preparation, probably represent direct effects of 100 μM concentrations of neuroleptics on the presynaptic membrane.

Comparison with results of other workers is somewhat confusing. Thus, Miller and Friedhoff [13], using rat striatal slices (2 × 3 mm), found that preincubation with low concentrations of haloperidol (10–100 nM) increased the K^+-evoked release of dopamine and the basal efflux was not affected, whilst higher concentrations (1–100 μM) reduced the K^+-evoked release and basal efflux was stimulated. Pimozide and chlorpromazine (1 μM) both increased the stimulation-induced overflow of [^{3}H]dopamine from neostriatal slices [14]. Preincubation of tissue with chlorpromazine (5 μM) also increased the stimulated-induced release of endogenous dopamine from rat striatal slices [15]. In contrast, preincubation with neuroleptics was shown to increase basal efflux of [^{3}H]dopamine with a corresponding inhibition in the electrically stimulated [12,16] or K^+-evoked release of [^{3}H]dopamine [5, 17], in agreement with the results reported here. The conflicting results may

be a consequence of the size of the tissue slices or the concentration of drugs used. Thus, in large slices (0.5–3 mm thick), activation of post-synaptic dopamine receptors, located on cholinergic interneurones [18], or the terminals of corticostriatal glutamatergic neurones [19] could result in feedback mechanisms with dopaminergic terminals, regulating dopamine release. Antagonism of muscarinic receptors by neuroleptics may also be important [20], although anti-muscarinic activity was shown to contribute very little to the action of neuroleptics in releasing [^{14}C]dopamine from striatal tissue [21].

The potency of equimolar concentrations of neuroleptics in enhancing the release of [^{3}H]dopamine showed a direct correlation with the lipophilicity of the drug (Fig. 3). Similarly, Seeman *et al.* [22] have shown the potencies of neuroleptics to block the conduction of rat sciatic and phrenic nerves, correlated with their octanol–H_2O partition coefficients. Neuroleptic drugs can lower the surface tension of Ringer's solution [23] and can generate a liquid membrane on a cellulose acetate membrane–water interface [24]. Since chlorpromazine (60–200 μM) severely damages the morphological state of catecholamine storage granules [25], the subsequent 'labilisation' of membranes could result in release of catecholamines from these structures. Therefore, these results, together with the present data, suggest that the enhanced release of dopamine by neuroleptics is associated with the ability of neuroleptics to interact with the lipid component of membranes. This mechanism for enhancing the release of dopamine is based solely on the lipophilicity of neuroleptics to 'fluidize' cellular and subcellular membranes. However, a barbiturate, methohexitone Na, which has similar octanol–H_2O partition coefficients to phenothiazines [26] did not enhance the release of dopamine. Therefore, although lipid solubility may be one determinant in the ability of 100 μM concentrations of neuroleptics to enhance release of dopamine, other factors are also involved. For example, phenothiazines can inhibit calmodulin-dependent events (calmodulin is an intracellular mediator of the effects of Ca^{2+} [27]) and the inhibition of stimulus-induced release of [^{3}H]dopamine may result from altered Ca^{2+} fluxes, thereby inhibiting the coupling between impulse and neurosecretion. In this respect, phenothiazines and structurally related antipsychotic drugs showed a good correlation between calmodulin inhibition and hydrophobicity, whereas drugs, such as pentobarbital, were only weak inhibitors of calmodulin [27].

The clinical relevance of these results is not known. The nmolar concentrations of neuroleptics, which inhibit binding of [^{3}H]haloperidol to striatal receptors and inhibit the stimulus-induced release of striatal [^{3}H]dopamine [12], show a good correlation with their clinical potencies and the concentrations found in the serum of patients using these drugs [28]. Clearly, the direct presynaptic effects of neuroleptics described in this study do not correlate with their clinical efficacy. However, chronic exposure to these drugs over a prolonged period may lead to accumulation in cellular and subcellular membranes of brain tissue. Chronic administration of chlorpromazine (33 mg/kg) for 14 days leads to 'partially damaged' rat heart mitochondria [25]. If μmolar concentrations (1–100 μM) are reached in the striatum, the transmitter releasing action may be involved in the extrapyramidal disorder, tardive dyskinesia, which seems to be due to over stimulation of cerebral dopamine receptors [29]. In this respect, the phenothiazines chlorpromazine, prochlorperazine and fluphenazine, which are very lipophilic and potent in enhancing dopamine release, are very prone to produce extrapyramidal side effects [30]. The phenothiazines with a piperidyl side chain, e.g. thioridazine and the benzamide derivatives, are the least lipophilic and are relatively poor in enhancing dopamine release, and these drugs show a low incidence of dyskinetic side effects. However, it is clear that further studies involving chronic administration of phenothiazines are required.

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