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COMMUNICATIONS

A comparison of *in vitro* and *in vivo* dopamine receptor antagonism produced by substituted benzamide drugs

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Cerebral dopamine receptor antagonism *in vivo* is associated with the ability to interact with two *in vitro* models of these receptors. Thus the ability to inhibit dopamine stimulation of the adenylate cyclase from rat striatum has been suggested as a model of post-synaptic dopamine receptor activity and of neuroleptic potential (Clement-Cormier, Kebabian & others, 1974; Miller, Horn & Iversen, 1974). Similarly, the ability to displace radioactive ligands from their binding sites in rat striatal preparations is also used as an index of an interaction with post-synaptic dopamine receptors and of neuroleptic activity (Seeman, Chau-Wong & others, 1975; Creese, Burt & Snyder, 1976). This latter model is believed to provide a better correlation between *in vivo* clinical neuroleptic activity and *in vitro* activity than the adenylate cyclase system.

We have previously reported that some substituted benzamide drugs, such as metoclopramide and sulpiride, exhibit behavioural and biochemical properties associated with a blockade of cerebral dopamine receptors (Dolphin, Jenner & others, 1975; Peringer, Jenner & Marsden, 1975; Peringer, Jenner & others, 1976; Elliott, Jenner & others, 1977). Such compounds to a variable degree block apomorphine-induced locomotor activity, inhibit apomorphine-induced circling in rodents with unilateral nigrostriatal lesions, and elevate striatal and mesolimbic concentrations of the dopamine metabolites homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC). We have, however, been unable to demonstrate a significant effect of these compounds on the dopamine stimulation of the adenylate cyclase from rat striatum. This, and other data, has led us to conclude that these compounds act on the cerebral dopamine pathways in a manner which differs from that of classical neuroleptics.

The present study is a comparison of the activity of five substituted benzamide drugs in the *in vitro* models of dopamine receptor activity with their ability to induce catalepsy and to inhibit apomorphine-induced stereotyped behaviour, these behavioural tests being chosen since they are often used to screen neuroleptic activity. These data are then compared with the properties of the compounds seen in clinical use with the object of determining whether the existing behavioural and biochemical models of dopamine receptor activity correlate for this group of drugs and if these properties relate to the clinical activity of the compounds.

The following substituted benzamide drugs have been investigated. Metoclopramide (*N*-[diethylaminoethyl]-2-methoxy-4-amino-5-chlorobenzamide hydrochloride), sulpiride (*N*-[1'-ethyl-2'-pyrrolidinylmethyl]-2-methoxy sulphamoyl benzamide), sultopride (*N*-[1-ethyl-2-pyrrolidinyl-methyl]-2-methoxy-5-ethylsulphonyl benzamide) and tiapride (*N*-[diethylaminoethyl]-2-methoxy-5-methylsulphonyl benzamide) were supplied by SESIF France. Clebopride as the malic acid salt (*N*-[*N'*-benzylpiperidin-4'-yl]-4-amino-5-chloro-2-methoxy benzamide) was supplied by Almirall Laboratories Ltd.

Dopamine-sensitive adenylate cyclase in the striatum from male Wistar rats (175–225 g; Animal Suppliers Ltd.) was measured using the method of Miller & others (1974). Dopamine (10^{-4}M) was used to produce a 2–3 fold increase in adenylate cyclase activity. Metoclopramide, sulpiride, sultopride, tiapride and clebopride were incorporated into the system in concentrations of 10^{-8}M to 10^{-4}M (as the base). Dopamine receptor binding was assayed in striatal preparations from female Wistar rats (150 g) by a modification of the technique of Creese, Burt & Snyder (1975). [^{3}H]Haloperidol (Janssen Pharmaceutica, Beerse, Belgium; 10 Ci mmol^{-1}) 2×10^{-9}M was used to label the striatal preparations. Sulpiride, metoclopramide, sultopride, tiapride and clebopride (10^{-10}–10^{-6}M) or vehicle were incorporated into the system. Incubates containing either (+)- or (−)- butaclamol (2×10^{-6} and 2×10^{-7}M respectively) were also included to distinguish specific and non-specific [^{3}H]haloperidol binding.

Catalepsy was assessed by placing the front paws of male Swiss S or P strain mice (20–25 g) over a 5 mm diameter metal bar raised 2 cm from bench level. Catalepsy was scored on a 0–6 system according to the time the animals remained in this position as previously described (Elliott & others, 1977). Tiapride (5–40 mg kg^{-1}) and sultopride (5–40 mg kg^{-1})-induced catalepsy was assessed at intervals up to 3 h after administration. Stereotypy was assessed in male Wistar rats (200–225 g) 15 min following apomorphine (0·5 mg kg^{-1}, s.c.).

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Metoclopramide (0·5–32 mg kg^{-1}), sulpiride (2–64 mg kg^{-1}), tiapride (2–64 mg kg^{-1}), sultopride (0·25–64 mg kg^{-1}) and clebopride (0·125–2·0 mg kg^{-1}) were administered 1 h before apomorphine administration. Data on the cataleptic activity and effect on adenylate cyclase for metoclopramide, sulpiride and clebopride have previously been reported but are presented here for comparative purposes.

Dopamine (10^{-4}M) stimulated adenylate cyclase activity approximately 3-fold. Basal concentrations were 29·1 ± 0·55 pmol cAMP/2 mg tissue wet weight per 2·5 min. In the presence of dopamine (10^{-4}M) activity was 81·6 ± 3·7 pmol cAMP/2 mg tissue wet weight per 2·5 min. The incorporation of sultopride or tiapride (10^{-8}–10^{-4}M) did not inhibit the stimulation of adenylate cyclase produced by dopamine ($P > 0·05$). These findings are in agreement with our previous data indicating that neither metoclopramide nor sulpiride have any inhibitory action on this system (Elliott & others, 1977). Clebopride (10^{-4}M) produced an approximate 20% inhibition of this system while lower concentrations were ineffective (Table 1).

Metoclopramide, sulpiride, sultopride and clebopride (10^{-10}–10^{-6}M) displaced [^{3}H]haloperidol from its binding site in rat striatal preparations (Table 1). Clebopride was the most potent in this respect but its activity was approximately 1/10th that of haloperidol (IC50 2·37 × 10^{-9}M—unpublished data obtained at the same time using the same experimental conditions). Tiapride in the concentrations examined was without effect.

The ability of most of the substituted benzamides to displace [^{3}H]haloperidol provides the first evidence of a direct effect of substituted benzamide drugs on cerebral dopamine receptors. Compared with classical dopamine antagonists, such as haloperidol, the interaction observed is weak. The lack of activity of tiapride is noteworthy since on other evidence it also appears to block cerebral dopamine receptors. Thus, tiapride elevates cerebral HVA and DOPAC concentrations and causes an inhibition of apomorphine-induced locomotor activity, stereotypy and circling behaviour (this study and unpublished data). However, clinically tiapride differs from the other substituted benzamides tested in having neither neuroleptic activity nor causing dyskinesias; on the contrary it is used for the treatment of abnormal movements (Table 1).

The administration of tiapride and sultopride to mice produced a very weak cataleptic activity (Table 1). The effect was maximal 2–3 h after drug administration in each case but the production of catalepsy showed no dose dependency between 10 and 40 mg kg^{-1}. These findings are similar to those for metoclopramide and sulpiride where again a weak cataleptic action with little evidence of dose dependency was apparent (Table 1). Clebopride, however, produced a pronounced dose-dependent cataleptic state.

Administration of apomorphine (0·5 mg kg^{-1}, s.c.) to rats produced an animal showing continuous sniffing, periodic locomotor activity and on occasions licking or biting. These phenomena were inhibited by metoclopramide, tiapride, sultopride and clebopride (Table 1). Sulpiride in doses up to 64 mg kg^{-1} was without effect (see also Puech, Simon & Boissier, 1976).

It is generally believed that substituted benzamide drugs act by inhibition of cerebral dopamine receptors as do the classical neuroleptic compounds. However, unlike this latter group, it seems that the substituted benzamides so far tested have little or no activity on the dopamine sensitive adenylate cyclase system from rat striatum. This might suggest either that (1) these compounds act on a dopamine receptor that is not dependent upon adenylate cyclase, or (2) that the receptor involved is dependent on adenylate cyclase but is located elsewhere in the brain, or (3) that adenylate cyclase activation is not an integral part of the cerebral dopamine receptor mechanism. Further, since two of the compounds tested are known to possess neuroleptic activity (sulpiride and sultopride) it appears that either the neuroleptic activity of these drugs is mediated by a mechanism differing from that of phenothiazine- and butyrophenone-type compounds, or that the interaction with striatal adenylate cyclase has little to do with neuroleptic activity. In this respect butyrophenone derivatives do not show as much activity in the adenylate cyclase model as would be expected from their neuroleptic activity (Miller & others, 1974). Such compounds

Table 1. *A comparison of the activity of some substituted benzamide drugs in* in vitro *models of dopamine receptor antagonism with their activity in* in vivo *behavioural models and their clinical characteristics.*

	IC50				Clinical characteristics**				
							Extrapyramidal side-effects		
Drug	Adenylate cyclase	[^{3}H]haloperidol binding	Catalepsy score at 40 mg kg^{-1}	ID50 stereotypy mg kg^{-1}	Anti-emetic	Neuroleptic	Acute dystonic reactions	Parkinsonism	Anti-dyskinetic activity
Tiapride	>10^{-4}M	>10^{-6}M	1·5	48	+	0	0	0	+
Metoclopramide	>10^{-4}M†	4·2 × 10^{-7}M	2·1†	5·4	+	0	+	0	0
Sulpiride	>10^{-4}M†	3·6 × 10^{-7}M	2·1†	>64	+	+	+	+	0
Sultopride	>10^{-4}M	1·8 × 10^{-7}M	2·4	41	+	+	+	+	0
Clebopride	>10^{-4}M†*	2·0 × 10^{-6}M	5·8†	0·34	+	?	?	?	?

† Previously published data (Elliott, Jenner & others, 1977).
* 20·8% inhibition at 10^{-4}M.
** assessed from published literature and unpublished data provided by SESIF France Ltd., and Almirall Laboratories Ltd.

are, however, more potent in displacing radioactive dopaminergic ligands from their binding sites in rat striatum. But even in this latter model the substituted benzamides are only weakly active. Clebopride, the most potent molecule of those tested, produces behavioural effects in animal experiments in doses equivalent to those of haloperidol. However, clebopride has only 1/10th the ability of haloperidol to displace the ligand in our experiments. While a good correlation exists between neuroleptic activity and activity in receptor binding models for classical neuroleptics, this would not appear to be the case for the substituted benzamides. Thus, while sulpiride and sultopride exhibit clinical neuroleptic activity their ability to displace [^{3}H]-haloperidol does not differ from that of metoclopramide which has no known neuroleptic properties.

Examination of the data from the *in vitro* models of dopamine receptor activity in terms of the production of extrapyramidal side effects by substituted benzamide drugs also fails to show any correlation. Thus, sultopride, which appears to produce an incidence of side effects approaching that of classical neuroleptics does not differ in activity from metoclopramide and sulpiride, which produce only a low incidence of such reactions. In view of the clinical observation that tiapride acts as an antidyskinetic agent, its inactivity in both *in vitro* models requires explanation.

The ability of these substituted benzamide drugs to inhibit apomorphine-induced stereotyped behaviour does not correlate with either their activity in displacing ^{3}[H]haloperidol or their clinical activity. For example, the lack of effect of sulpiride in inhibiting stereotyped behaviour compared with the fairly potent action of metoclopramide contrasts with their equivalence in displacing [^{3}H]haloperidol. The same comparison shows the lack of neuroleptic activity of metoclopramide in comparison with its activity in inhibiting stereotyped behaviour whereas the reverse is true for sulpiride. Indeed, the data in Table 1 could be interpreted to indicate that the neuroleptic activity of substituted benzamide drugs is associated with a failure to inhibit stereotyped behaviour.

The ability of substituted benzamide drugs to interact with the *in vitro* models of dopamine receptor activity does, however, correlate with their cataleptic activity. Thus, clebopride, which was the only compound to inhibit dopamine stimulation of the adenylate cyclase system and was the most potent compound in displacing [^{3}H]haloperidol, was also the only benzamide tested to show conspicuous dose-dependent production of catalepsy. The equivalent activity of metoclopramide, sulpiride and sultopride in displacing [^{3}H]haloperidol correlated with their equal albeit weak cataleptic potency, whilst tiapride was less active behaviourally and biochemically. Cataleptic activity does not, however, correlate with clinical neuroleptic activity or with the production of extrapyramidal side-effects. Thus the equivalent cataleptic activity of metoclopramide, sulpiride and sultopride is associated with both a lack of and presence of neuroleptic activity and extrapyramidal side effects.

In conclusion it would appear that for these substituted benzamide drugs there is little relation between the behavioural and biochemical models of dopamine receptor antagonism employed and their clinical properties. Indeed it may well be that the activity of dopamine receptor antagonists in *in vitro* models of striatal neuronal activity is associated more with the production of catalepsy (and other behaviours) than with neuroleptic activity and that the good correlation found with classical neuroleptics is coincidentally related to their ability to induce cataleptic states.

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