Differential activities of some benzamide derivatives on peripheral and intracerebral administration

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The benzamide group of drugs has been classified with the neuroleptic agents, and there is evidence to suggest that agents from the series are able to inhibit dopamine function. Indeed, a common characteristic of the benzamides is their ability to affect dopamine systems outside the blood-brain barrier, for example, in the pituitary gland, area postrema or in the stomach (Fang, Zimo & Byyny, 1977; Horowsky & Graf, 1976; Jenner, Elliott & others, 1978; Prieto, Moragues & others, 1977; Valenzuela, 1976). However, the central activity spectra of the benzamides differ markedly when the agents are administered by a peripheral route (Jenner & others, 1978), and this may reflect differing abilities to penetrate cerebral tissue. The present studies were, therefore, designed to compare against an amphetamine-induced hyperactivity, the antagonistic effects of metoclopramide, sultopride, clebopride, tiapride and sulpiride when administered peripherally (i.p.) or intracerebrally. Fluphenazine was used as the control neuroleptic and the site of intracerebral administration was established by determining its anti-amphetamine effects after injection into several brain regions.

Male Sprague-Dawley rats (250–300 g) which were prepared for intracerebral injection by stereotaxically implanting guide cannulae (0·64 mm diameter stainless steel tubing) for bilateral administration into the nucleus accumbens, Ant. 9·4, Vert. +2·5 (2·5 mm), Lat. ±1·6, the tuberculum olfactorium, Ant. 9·0, Vert. +4·0 (6·7 mm), Lat. ±2·5, the amygdala, Ant. 5·6, Vert. +2·0 (3·7 mm), Lat. ±4·5, the caudate-putamen, Ant. 8·0, Vert. +3·0 (1·5 mm), Lat. ±3·0, the cerebral cortex, Ant. 8·0, Vert. +5·5 (1·0 mm), Lat. 3·5, and into the midbrain, Ant. 1·0, Vert. +0·5 (3·0 mm), Lat. ±1·5, or unilateral injection into the lateral ventricles, Ant. 8·0, Vert. +4·0 (2·0 mm), Lat. −1·5 (De Groot, 1959). Animals were used once, 14 days after surgery when stainless steel stylets (0·3 mm diameter), which kept the guides patent, were replaced by injection units made of the same tubing and extending below the guides by a distance indicated in parentheses with the vertical coordinates above. Drug was delivered from micrometer syringes in a volume of 1 μl over 60 s, the animals being manually restrained. The locations of the guide cannulae were confirmed histologically.

Hyperactivity was induced by amphetamine 1·5 mg kg^{-1}, i.p. (preliminary studies show this dose produces a maximal hyperactivity in the absence of a stereotyped behaviour which would interfere with the recording of hyperactivity). Rats were placed in Perspex cages (30 × 20 cm and 15 cm high) each fitted with one photocell unit placed off-centre. Hyperactivity was recorded electromechanically as the number of interruptions of the light beam occurring in 5 min. This hyperactivity induced by amphetamine was shown to be inhibited by fluphenazine injected into the nucleus accumbens. A brief inhibition was observed after a delay of 30 min when fluphenazine was injected into the lateral ventricles, and a brief and modest reduction was recorded after intracaudate fluphenazine, but only after a delay of 90 min. The amphetamine response was not modified when fluphenazine was injected into the tuberculum olfactorium, amygdala or midbrain, and was significantly enhanced 90–120 min after injection into the cerebral cortex (Table 1). Therefore, the nucleus accumbens was selected as a suitable site for injection of the benzamide derivatives to determine their anti-amphetamine effects.

All behavioural studies were in a sound-proofed, diffusely illuminated room maintained at 21 ± 2°. (+)-Amphetamine SO_4 (Sigma), fluphenazine HCl (Squibb), metoclopramide monohydrochloride (Beecham), sultopride HCl (Delagrange), clebopride HCl (Almirall) and tiapride HCl (Delagrange) were prepared for peripheral and intracerebral injection in distilled water. Sulpiride (Delagrange) was similarly prepared for intra-accumbens injection: a minimum quantity of hydrochloric acid was used to prepare the solution for intraperitoneal injection.

Table 1. *Effect of fluphenazine* (1·25 μg) *injected bilaterally into different brain regions on the hyperactivity induced by amphetamine* (1·5 *mg* kg^{-1}, *i.p.*).

Time after inj. (min)	Amphetamine hyperactivity (counts/5 min) following fluphenazine inj. into brain areas							
	None	ACB	TUO	AM	CP	CC	MB	VT
15	65	13**	57	67	62	64	59	66
30	70	0**	63	74	52	72	68	61
45	70	0**	59	62	50	89	71	15**
60	58	0**	66	60	49	84	68	18**
90	52	2**	61	65	37	68	55	47
120	41	0**	50	49	20*	68†	60	52
150	34	1**	45	49	23	66†	46	45
180	33	0**	45	33	10*	53†	31	46

Amphetamine and fluphenazine were administered together. Fluphenazine was injected bilaterally into the ACB (nucleus accumbens), TUO (tuberculum olfactorium), AM (amygdala), CP (caudate-putamen), CC (cerebral cortex) or MB (mid-brain). A unilateral injection of 2·5 μg fluphenazine was also made into the lateral ventricles (VT). Control animals (none) received amphetamine only. There were 5–7 rats in each group and s.e.s of the means were in the range 10–23%. Significant reductions in amphetamine hyperactivity are indicated by $*P<0.05$, $**P<0.001$, and significant increases by $\dagger P<0.05$.

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Table 2. *Effect on amphetamine hyperactivity of fluphenazine and some benzamide derivatives injected peripherally or into the nucleus accumbens.*

Drug	Peripherally Dose mg kg^{-1}	Peripherally % inhibition amphetamine hyperactivity	Nucleus accumbens Dose µg	Nucleus accumbens % inhibition amphetamine hyperactivity
Fluphenazine	0·01	0		
	0·04	40	0·2	2
	0·1	90	0·4	17
	0·4	100	0·8	100
Metoclopramide	0·4	4		
	1·0	30	20·0	7
	4·0	60	40·0	20
	10·0	94	80·0	50
Sultopride	0·4	0		
	1·0	21	0·625	22
	4·0	40	1·25	60
	10·0	100	2·5	94
Clebopride	0·04	0		
	0·1	14	2·5	6
	0·4	43	5·0	41
	1·0	88	10·0	86
Tiapride	1·0	0		
	4·0	0	0·31	5
	10·0	0	0·625	63
	40·0	18	1·25	96
Sulpiride	1·0	0		
	4·0	0	1·25	8
	10·0	0	2·5	74
	40·0	0	5·0	97

Amphetamine and the potential antagonists were administered together, amphetamine (1·5 mg kg^{-1}) by the i.p. route and the antagonists i.p. (mg kg^{-1}) or directly into the nucleus accumbens (µg). The effects of fluphenazine, sultopride and clebopride were significant by both routes ($P<0·05$–$P<0·001$). Tiapride and sulpiride significantly ($P<0·001$) antagonised amphetamine hyperactivity only when administered intra-accumbens. Metoclopramide was more active by the peripheral ($P<0·05$–$P<0·001$) than by the intra-accumbens route ($P<0·05$ only). s.e.m.s for metoclopramide were large (+20%) whilst for other agents s.e.m.s were in the range 9–17%. There were 6–8 rats in each group. Intra-accumbens solvent failed to antagonise amphetamine hyperactivity.

When administered peripherally, sulpiride (1–40 mg kg^{-1}) failed to reduce amphetamine hyperactivity although 1·25–5·0 µg administered into the nucleus accumbens caused a dose-dependent antagonism of the amphetamine response, with the larger dose producing a virtually complete inhibition. Similar observations were made when tiapride was used, it had only a weak anti-amphetamine effect in a dose as large as 40 mg kg^{-1} i.p., but caused a dose-dependent and marked antagonism of amphetamine when administered directly into the nucleus accumbens in even lower doses than sulpiride (0·31–1·25 µg) (Table 2). Metoclopramide, sultopride and clebopride (as well as fluphenazine) were active against amphetamine both by the i.p. and intra-accumbens routes (Table 2).

Sulpiride and tiapride exert only weak effects in many tests for neuroleptic action in the brain (Costall & Naylor, 1975; Tagliamonte, De Montis & others, 1975; Costall, Funderburk & Naylor, in preparation). and this is further emphasized by present observations that, on peripheral administration, both drugs fail to antagonize amphetamine-induced hyperactivity. Further, in those tests where peripherally administered sulpiride or tiapride are effective, for example, when they antagonize the hyperactivity induced by dopamine injected into the nucleus accumbens or caudate-putamen (Costall & Naylor, 1976), they are much less potent than clebopride or the classical neuroleptics such as fluphenazine. The present results indicate that the low efficacy of sulpiride or tiapride on peripheral administration may partly relate to a reduced ability to penetrate the blood-brain barrier. Thus, whilst the anti-amphetamine activities of clebopride and sultopride were in contrast to the ineffectiveness of sulpiride or tiapride when these drugs were administered peripherally, *all* agents exhibited anti-amphetamine effects when injected directly into the nucleus accumbens. This conclusion is in agreement with the interpretation of Honda, Satoh & others (1977) who found that sulpiride injected into the cerebral ventricles was virtually equipotent with haloperidol in inhibiting apomorphine and amphetamine-induced behaviour.

We do not exclude an action of the benzamide derivatives in brain areas other than the nucleus accumbens, particularly after peripheral administration. We have previously postulated a minor role for the nucleus accumbens in the action of metoclopramide in reducing a dopamine hyperactivity (Costall & Naylor, 1976) and it is therefore of interest that intra-accumbens metoclopramide was required in very high doses, which may easily diffuse, to reduce amphetamine hyperactivity. Whether or not diffusion does occur for example, to the tuberculum olfactorium or caudate-putamen, the fundamental observation remains that whilst sulpiride and tiapride are only weakly active given intraperitoneally, given intracerebrally they are as potent as fluphenazine in inhibiting amphetamine hyperactivity.

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Effect of chronic amphetamine administration on the behaviour of rats in the open field apparatus: reversal of post-withdrawal depression by two antidepressants

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The chronic administration of low doses ($<1{\cdot}0$ mg kg^{-1}) of (+)-amphetamine to rats results in hyperactivity. Higher doses of the drug ($>1{\cdot}5$ mg kg^{-1}) lead to marked behavioural stereotypy (Randrup, Munkvad & Udsen, 1963; Ellinwood, Sudilovsky & Nelson, 1973; Ellinwood & Balster, 1974). However, apart from the observations of Tonge (1974) the changes in behaviour of rats following the withdrawal of amphetamine have received scant attention. We have investigated the changes in four behavioural parameters measured in the 'open field' apparatus of groups of rats chronically treated with amphetamine for 21 days and then at 2, 4 and 6 days after drug withdrawal. As the ambulation and rearing activity of the rats was markedly decreased following amphetamine withdrawal we then investigated the effects of three types of antidepressant drugs, which had been administered for several days, on the behaviour of the depressed animals. Such a study, though preliminary, may lead to the development of a model for the evaluation of potential antidepressant drugs.

Mature male Wistar rats were housed in groups of 5 to a cage and had free access to food and drink for the duration of the experiment. 4 experimental groups were treated with (+)-amphetamine, administered in their drinking water, for 21 days. The drug doses increased from 50 mg litre^{-1} after the third day to 100 mg litre^{-1} until day 14 and finally to 200 mg litre^{-1} for the third week. An equal weight of ascorbic acid was added to serve as an antioxidant. The control groups received ascorbic acid only in their drinking water. The water bottles were covered with dark paper and the freshly-prepared drug solution was replaced every two days.

On day 21 the animals were withdrawn and on day 22 the first of 6 daily treatments of an antidepressant agent was administered intraperitoneally. Each group received one of three antidepressants amitriptyline (10 mg kg^{-1}), mianserine (15 mg kg^{-1}) or pargyline (25 mg kg^{-1}). The control groups received an equal volume of vehicle (0·9% w/v NaCl).

During the experimental period the animals' behavioural patterns were assessed at regular intervals using the 'open field' apparatus of the type described by Gray, Levine & Broadhurst (1965) and Gray & Lalljee (1974). Behavioural observations were made at the same time each day. Each animal was tested only once to prevent habituation. This was essential as habituation to the 'open field' leads to the animals showing almost complete inactivity. The walls and base of the apparatus were cleaned with distilled water after each 3-min 'open field' test. Four behavioural parameters were measured: ambulation, rearing grooming and defaecation. The Student's *t*-test was used to evaluate the data. The alpha level was chosen as 0·05.

The results of this study are shown in Tables 1 and 2.

Both amitriptyline and mianserine when given alone significantly reduced the ambulation and defaecation scores (Table 1). Mianserin and pargyline alone also reduced the rearing scores. In those animals which had been treated with amphetamine for 3 weeks before withdrawal, the fluid intake did not differ significantly from that of the control group (control 30 ± 4 ml day^{-1}, experimental 28 ± 3 ml day^{-1}). Nevertheless, the weight of the test group was significantly lower than that of the controls ($P<0{\cdot}05$) (controls 290 ± 15 g, experimental $250^{*} \pm 10$ g) at the end of the amphetamine treatment. The ambulation scores increased throughout the period of amphetamine administration reaching a peak on the day the drug was withdrawn. Neither the rearing nor the grooming scores were significantly different from the control value at the end of the treatment. On withdrawal of the drug, a

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