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MAZINDOL ANOREXIA IS MEDIATED BY ACTIVATION OF DOPAMINERGIC MECHANISMS

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1 Anorexia in rats following injections of mazindol (0.1–8 mg/kg i.p.) could be antagonized by pretreatment with a dopamine receptor blocker (pimozide) but not by pretreatment with an α-adrenoceptor blocker (phenoxybenzamine), a β-adrenoceptor blocker ((−)-propranolol), or a 5-hydroxytryptamine receptor blocker (methergoline).

2 In rats with a unilateral lesion in the substantia nigra made by stereotaxic injection of 6-hydroxydopamine, mazindol caused a dose-dependent turning towards the lesioned side, indicating an indirect mechanism of action. This effect could be antagonized by pretreatment with a dopamine receptor blocker.

3 In rats pretreated with reserpine and α-methyl-*p*-tyrosine, mazindol did not have any motor stimulant action.

4 *In vitro* studies with synaptosomes prepared from rat brain, indicated that mazindol blocks uptake and causes release of dopamine.

5 It is concluded that the anorectic action of mazindol is mediated by a dopaminergic mechanism.

Introduction

Mazindol has recently been introduced as an anorectic, and it differs from all other previously available anorectics in that it is not structurally related to amphetamine. Gogerty, Penberthy, Iorio & Trapold (1975) have described it as having weak central nervous system stimulant properties with marked anorectic activity. Gogerty *et al.* (1975) and Engstrom, Kelly & Gogerty (1975), suggest that the anorectic activity of mazindol is due to its ability to block neuronal uptake of noradrenaline. As noradrenaline is known to stimulate rather than inhibit feeding when applied intracerebrally (Booth, 1968; Slangen & Miller, 1969), while dopamine and 5-hydroxytryptamine (5-HT) inhibit feeding when applied by this route (Kruk, 1973), it was decided to examine the activity of specific receptor antagonists on the anorectic response to mazindol. In the event, dopamine receptor stimulation was indicated, and the activity of mazindol in other dopamine-sensitive animal models *in vivo* was examined. Finally, *in vitro* studies of uptake and release of dopamine using neostriatal synaptosomes were made to confirm the evidence obtained by the *in vivo* studies.

Methods

Measurement of anorectic response in rats

Groups of six adult Sprague-Dawley rats were housed in a room maintained at 20°C ± 1°C and a 12 h light, 12 h dark cycle, the light cycle starting at 07 h 00 min. The rats were trained to eat their daily food intake in a period of 4 h, and the amount eaten after 1, 2 and 4 h was recorded. Water was available at all times. All drugs were injected intraperitoneally, and the doses refer to the form listed below. Anorectic drugs were always administered at 11 h 30 min, which was 30 min before food presentation at 12 h 00 min. Pretreatment times refer to the time before the start of the feeding period. Inhibition of feeding was expressed as the percentage decrease in food eaten in the first hour following drug treatment, when compared with appropriate saline (0.9% w/v NaCl solution) control for that group on the previous day. ED_{50} values (defined as the dose of drug required to decrease the food eaten by 50% in the first hour) were calculated by the method of Litchfield & Wilcoxon (1949). At least five groups of six animals were always used to determine any dose-response curve.

Unilateral injections of 6-hydroxydopamine

Male rats weighing 160–180 g were anaesthetized with sodium methohexitone (Brietal, Eli Lilly) 50 mg/kg, and placed in a David Kopff stereotaxic frame. A unilateral injection of 6-hydroxydopamine (25 μg/rat) in 5 μl was made at coordinates A 2.4, L 1.7, V 1.9–2.9 above the intra-aural line, which is in the region which includes the zona compacta of the substantia nigra (König & Klippel, 1967).

Measurement of turning activity

Turning activity was measured by direct observation of individual rats placed in an open field measuring 57 cm × 57 cm. Counts were made for 1 min at the times indicated in the figure. Spontaneous locomotor activity was observed in the same open field.

Measurement of [^{3}H]-dopamine uptake and release by brain synaptosomes

Uptake. Uptake studies were made on synaptosomes by a method based on that of Snyder & Coyle (1969). The striata from male rats were homogenized with a teflon pestle in a glass grinding vessel in 8 volumes of 0.25M sucrose. Homogenates were centrifuged at 1000 *g* for 10 min at 4°C, and 100 μl of the supernatant synaptosomal fraction were then preincubated with shaking at 37°C for 5 min in an atmosphere of 95% O_2 and 5% CO_2, in a final volume of 2 ml. The incubation medium was Krebs-Henseleit solution of the following composition (mM) NaCl 117, KCl 5.3, $MgSO_4$ 0.53, $CaCl_2$ 0.95, NaH_2PO_4 0.90, $NaHCO_3$ 25.0, glucose 11.1, ascorbic acid 1.13, disodium edetate 0.13 and pargyline 0.125. After preincubation, [^{3}H]-dopamine was added to make a final concentration in the incubation medium of 0.1 μM. Incubation was continued for 10 min under conditions identical to those of the preincubation. Incubation was stopped by rapid cooling in an ice bath, and the tissue and incubation medium were separated by centrifugation at 50,000 *g* for 30 min at 1–2°C. A sample (0.1 ml) of the supernatant fluid was taken and total radioactivity determined by liquid scintillation counting. The remaining supernatant was discarded and the pellet was rinsed twice with 2 ml ice cold saline. Absolute ethanol (1 ml) was added to the tube containing the pellet, and left to extract at 1–2°C for 16 h; 0.1 ml of the ethanol was taken for liquid scintillation counting. Particle medium ratios were calculated as counts min^{-1} g wet $tissue^{-1}$ divided by counts min^{-1} ml incubation $medium^{-1}$. Blank values from samples identically prepared and treated but incubated at 1–2°C were subtracted and inhibition of uptake was calculated by comparison with samples incubated free of drug. IC_{50} values, defined as the molar concentration of drug producing 50% inhibition of [^{3}H]-dopamine uptake in 10 min were calculated by plotting the % inhibition of uptake against concentration of inhibitor on semilogarithmic paper. At least six determinations were made at each point.

Release. The ability of mazindol to release [^{3}H]-dopamine from synaptosomes was studied by a method based on that of Ferris, Tang & Maxwell (1972). Synaptosomes from rat striatum were prepared as described above; 3 ml of the 1000 *g* supernatant fluid containing synaptosomes was incubated with 17 ml incubation medium (composition described above), at 37°C in an atmosphere of 95% O_2 and 5% CO_2, with shaking, for 10 minutes. [^{3}H]-dopamine (final concentration 0.1 μM) was then added, and incubation continued for 5 minutes. The incubate was then cooled to 1–2°C and centrifuged at 50,000 *g* for 30 min at 1–2°C. The supernatant fluid was discarded, and the pellet was carefully washed twice with 2 ml ice cold saline. The pellet was then resuspended in the original volume (3 ml) of ice cold incubation medium; 100 μl of this preparation was then added to 1.9 ml fresh incubation medium (preincubated for 5 min at 37°C in 95% O_2 and 5% CO_2) and incubation continued for a further 5 min in either the presence or absence of drug. Incubation was stopped by rapid cooling, and particle : medium ratios were determined as described above. The percentage [^{3}H]-dopamine released in the absence of drug was subtracted from the percentage [^{3}H]-dopamine released in the presence of drug. The corrected percentage of [^{3}H]-dopamine released under the influence of drug alone was then plotted against concentration of drug on semilogarithmic paper, and RC_{50} values (defined as the molar concentration of drug required to release 50% of the [^{3}H]-dopamine from the synaptosomes in 5 min) were determined.

Drugs

The following drugs were used: mazindol (Wander), (+)-amphetamine sulphate, phenoxybenzamine hydrochloride, (SK & F), apomorphine hydrochloride (MacFarlane Smith), pimozide (Janssen), 6-hydroxydopamine dihydrobromide, DL-α-methyl-*p*-tyrosine methyl ester (Sigma), (–)-propranolol (ICI), methergoline (Pharmatalia), reserpine (BDH), pargyline (Abbott), [^{3}H]-dopamine hydrochloride (5.6 Ci/mmol) (The Radiochemical Centre, Amersham).

Results

Antagonism of anorectic responses to mazindol by receptor blockers

Dose-effect relationships were established for mazindol-induced anorexia in the absence (saline pretreatment) or presence of drugs with receptor blocking

activity. From these dose-effect curves ED_{50} values were calculated by the method of Litchfield & Wilcoxon (1949).

Following pretreatment with pimozide, a significant antagonism of mazindol anorexia was seen, and the ED_{50} values were significantly increased. 5-HT receptor blockade, α-adrenoceptor block or β-adrenoceptor block did not antagonize mazindol anorexia (Table 1).

Whereas pimozide and methergoline had no effect, (−)-propranolol (10 mg/kg) and phenoxybenzamine (5 mg/kg) decreased food intake (Table 2). The ED_{50} for mazindol following propranolol was significantly decreased; after phenoxybenzamine pretreatment the ED_{50} value for mazindol was not significantly changed.

While the receptor antagonist experiments indicated that mazindol interacts with dopamine receptors, it was not possible to distinguish from feeding experiments whether the action was by direct receptor stimulation, or indirectly by release and/or block of re-uptake.

Animal models used to distinguish between direct and indirect receptor activation

Turning in rats with a unilateral substantia nigra lesion. Mazindol caused a dose-dependent turning towards the lesioned side, and this effect could be antagonized by pretreatment with pimozide (Figure 1). In the same rats, (+)-amphetamine (1–8 mg/kg i.p.) caused a dose-dependent turning towards the lesioned side, while apomorphine (0.1–1.0 mg/kg s.c.) caused a dose-dependent turning away from the lesioned side. In the conventional interpretation of this model (Ungerstedt, 1971) direct receptor stimulant drugs such as apomorphine cause turning away from the lesioned side due to direct stimulation of supersensitive receptors, while indirect receptor stimulant drugs such as amphetamine, cause turning towards the lesioned side by releasing dopamine from the unlesioned side. By analogy in this particular model, it was concluded that mazindol acts indirectly in a manner similar to that of (+)-amphetamine.

Table 1 Effect of receptor blockers on mazindol-induced anorexia

Pretreatment (mg/kg i.p.)	*Pretreatment time (h)*	*Treatment range of 5 or 6 doses (mg/kg i.p.)*	*ED_{50} (±95% confidence limits) (mg/kp i.p.)*	P
Saline	2	Mazindol (0.5–8)	2.4 (5.25–1.10)	
Pimozide 0.062	2	Mazindol (1–16)	5.1 (11.22–2.32)	<0.05
Pimozide 0.125	2	Mazindol (1–16)	5.8 (12.06–2.79)	<0.05
Pimozide 0.25	2	Mazindol (1–16)	6.9 (10.42–4.57)	<0.05
Methergoline 0.5	2	Mazindol (0.5–8)	2.0 (4.58–0.87)	NS
Phenoxybenzamine 5	1	Mazindol (0.5–8)	2.4 (4.12–1.4)	NS
Propranolol 10	1	Mazindol (0.5–8)	0.98 (1.47–0.65)	<0.05

ED_{50} values are based on the food eaten during the first hour after presentation of food, 30 min after mazindol treatment.

Table 2 Effect of receptor blockers on feeding in groups of 6 trained rats

Pretreatment (Saline 5 ml/kg or receptor blocker mg/kg i.p.)	*Pretreatment time (h)*	*Food eaten in the first hour after saline pretreatment (g ± s.e. mean, n = 6)*	*Food eaten in the first hour after receptor blocker pretreatment (g ± s.e. mean, n = 6)*	P
Pimozide 0.125	2	71.5 ± 4.6	67.8 ± 3.0	NS
Pimozide 0.25	2	70.5 ± 2.6	75.0 ± 2.5	NS
Methergoline 0.5	2	60.8 ± 5.8	66.0 ± 3.5	NS
Phenoxybenzamine 5	1	60.7 ± 4.5	46.7 ± 4.2	<0.05
(−)-Propranolol 10	1	71.4 ± 4.7	57.5 ± 4.9	<0.05

Saline, 5 ml/kg was administered 30 min before presentation of food, after the pretreatments as shown.

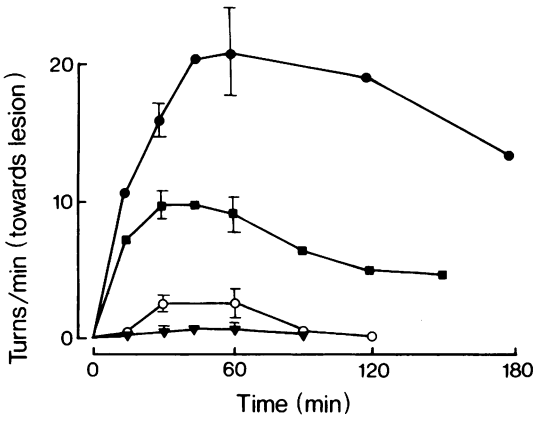

Figure 1 Effect of mazindol (○) 2 mg/kg, (■) 4 mg/kg, (●) 8 mg/kg i.p., and mazindol (▲) 4 mg/kg i.p. 2 h after pimozide 0.5 mg/kg, on turning behaviour in rats with a unilateral 6-hydroxydopamine lesion in the substantia nigra. $n = 6$. Vertical bars indicate s.e. mean.

Spontaneous activity in reserpine and α-methyl-p-tyrosine treated animals. By pretreatment with reserpine (5 mg/kg) 16 h previously and DL-α-methyl-*p*-tyrosine (250 mg/kg) 1 h previously, it is possible to test drugs in animals in which storage and synthesis of catecholamines is severely decreased (Andén, Fuxe, Hökfelt & Rubensson (1967). As shown in Table 3, the response of animals treated with mazindol was indistinguishable from the response observed following (+)-amphetamine. The effect could not be altered by increasing the dose of either mazindol or (+)-amphetamine. By contrast, the direct dopamine receptor stimulant apomorphine caused a dose-dependent increase in motor activity, aggression and chewing activity, some of the animals inflicting major damage to their own hind legs and tails by their compulsive chewing.

The results from this series of experiments indicated that mazindol has indirect dopamine receptor stimulant action, and it was therefore decided to investigate the ability of mazindol to release and block re-uptake of dopamine.

Effect of mazindol on uptake and release of dopamine in striatal synaptosomes

Mazindol blocked the uptake of [^{3}H]-dopamine into synaptosomes prepared from rat neostriatum in a concentration-dependent manner (Table 4). At a concentration 50 times greater than that needed to block uptake of dopamine by 50%, mazindol could release 50% of the [^{3}H]-dopamine previously taken up into neostriatal synaptosomes. For comparative purposes, (+)-amphetamine was also tested in this system and it is seen that amphetamine is approximately 3 times more potent as an uptake blocker and 300 times more potent as a releaser of dopamine than mazindol.

Discussion

Mazindol anorexia was antagonized by a specific dopamine receptor blocking compound, pimozide (Andén, Butcher, Corrodi, Fuxe & Ungerstedt, 1970). Pimozide also antagonizes (+)-amphetamine-induced anorexia (Kruk, 1973). The antagonism of the

Table 3 Effect of mazindol, (+)-amphetamine and apomorphine on spontaneous behaviour in rats, pretreated 16 h previously with reserpine (5 mg/kg i.p.) and 1 h previously with DL-α-methyl-*p*-tyrosine (250 mg/kg i.p.)

Mazindol (4 mg/kg i.p.)	*(+)-Amphetamine (4 mg/kg i.p.)*	*Apomorphine (1 mg/kg s.c.)*
Still, hunched, 'reserpinized'	Still, hunched, 'reserpinized'	Aggressive, vocal, difficult to handle, self-chewing

Six rats/group.

Table 4 Effect of mazindol on the uptake and release of [^{3}H]-dopamine by synaptosomes prepared from rat neostriatum

Drug	*IC_{50} uptake*	*RC_{50} release*
Mazindol	0.27 ± 0.04 μM (6)	70 ± 3.5 μM (7)
(+)-Amphetamine (base)	66 ± 15 nM (7)	0.25 ± 0.22 μM (6)

anorectic response by pimozide could be overcome by increasing the dose of mazindol, thus showing that the antagonism is at dopamine receptors, is competitive and reversible. This is in agreement with the finding of Barzaghi, Groppetti, Mantegazza & Müller (1973) and Kruk (1973), who found that stimulation of dopamine receptors with a direct receptor agonist, apomorphine, produced anorexia. These authors all concluded that activation of dopamine receptors can mediate anorectic responses.

Activation of 5-HT receptors by direct application of 5-HT (Kruk, 1973) or indirectly by release of 5-HT by drugs such as fenfluramine and norfenfluramine (Clineschmidt, McGuffin & Werner, 1974; Broekkamp, Weemaes & Van Rossum, 1975) can produce anorexia in rats. In the experiments reported here, methergoline, a specific 5-HT receptor blocker (Beretta, Ferrini & Glässer, 1965), did not antagonize the anorectic response to mazindol; thus it was concluded that activation of 5-HT mechanisms is not involved in mediating mazindol anorexia.

Engstrom *et al.* (1975) and Gogerty *et al.* (1975) tentatively suggest that mazindol anorexia may be associated with the ability of mazindol to block noradrenaline uptake, presumably implying that the anorexia is mediated by activation of noradrenaline-sensitive receptors. The lack of antagonism by α-adrenoceptor of β-adrenoceptor blocking agents in the present experiments, argues against noradrenaline having a role in mediating anorectic responses to mazindol. Schmitt (1973) found that neither α-adrenoceptor nor β-adrenoceptor blocking agents antagonized anorectic responses to (+)-amphetamine or fenfluramine, indeed it would be unexpected to find noradrenaline having a role in mediating anorectic responses as it is well documented that direct application of noradrenaline or substances stimulating α-adrenoceptors, stimulates feeding in satiated rats, and this effect can be antagonized by α-adrenoceptor blocking agents (Booth, 1968; Broekkamp & Van Rossum, 1972; Kruk, 1973).

From the anorexia antagonism studies with specific receptor blockers, it was concluded that mazindol exerts its action through activation of dopamine receptors. Whether this effect is due to a direct or indirect action on these receptors, was initially investigated *in vivo*.

When mazindol was tested in the substantia nigra-lesioned turning rat model (Ungerstedt, 1971) a dose-dependent turning towards the lesioned side was observed. This turning behaviour could be antagonized by pretreatment with pimozide; thus both turning behaviour and anorexia in response to mazindol are mediated by dopamine receptor activation. The direction of turning, namely towards the lesioned side, is the same as is observed with indirectly acting dopamine receptor agonists such as amphetamine which suggests that mazindol has indirect receptor stimulant activity. This conclusion was corroborated by experiments done in rats in which monoamine stores had been depleted with reserpine, and catecholamine synthesis had been inhibited with α-methyl-*p*-tyrosine. In this model, the direct dopamine receptor stimulant apomorphine produced exaggerated behavioural stimulation, while mazindol, in common with (+)-amphetamine, was inactive.

The indirect dopamine receptor stimulant activity of mazindol *in vivo*, was confirmed by biochemical studies *in vitro*. Mazindol effectively blocked the uptake of [^{3}H]-dopamine into synaptosomes prepared from rat neostriatum, the inhibition being concentration-dependent. Further, at concentrations higher than those needed to block dopamine uptake *in vitro*, a marked release of [^{3}H]-dopamine from synaptosomes was observed.

Evidence from *in vivo* experiments using mazindol and specific receptor blockers suggests that it has indirect dopamine receptor stimulant properties. *In vitro* experiments show that mazindol causes the release and blocks the uptake of dopamine. From these two lines of evidence it is concluded that the anorectic effect of mazindol is probably mediated by activation of a dopaminergic mechanism.

Note added after submission of manuscript

After submission of this manuscript, articles by Carruba, M.O., Groppetti, A., Mantegazza, P., Vicentini, L. & Zambotti, F. (1976). *Br. J. Pharmac.*, **56**, 431–436, and Zambotti, F., Carruba, M.O., Barzaghi, F., Vicentini, L., Groppetti, A. & Mantegazza, P. (1976). *Eur. J. Pharmac.*, **36**, 405–412, have appeared, which agree with our conclusions that behavioural and biochemical actions of mazindol are mediated by dopaminergic mechanisms.

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