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EFFECTS OF MAZINDOL, A NON-PHENYLETHYLAMINE ANOREXIGENIC AGENT, ON BIOGENIC AMINE LEVELS AND TURNOVER RATE

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1 Mazindol is a new anorexigenic agent which possesses a different chemical structure from that of phenylethylamines, but shows a pharmacological profile similar to that of (+)-amphetamine.

2 Mazindol neither altered whole brain monoamine levels (noradrenaline (NA), dopamine, 5-hydroxytryptamine (5-HT)) nor changed NA levels in the hypothalamus or dopamine levels in the caudate nucleus.

3 Mazindol enhanced dopamine turnover rate in the caudate nucleus, as shown by the increased rate of dopamine decline after blockade of catecholamine synthesis by α-methyl-*p*-tyrosine and decreased the conversion index of [^{3}H]-tyrosine into brain NA.

4 Mazindol administration did not modify pargyline-induced decline of 5-hydroxyindoleacetic acid suggesting that 5-HT turnover is not altered by this drug.

Introduction

Food intake can be suppressed by drugs, such as fenfluramine and (+)-amphetamine which have many diverse behavioural and biochemical properties but are both anorectics. Considerable evidence suggests that the anorectic effect of (+)-amphetamine is mediated by interactions with brain catecholamines (Weissman, Koe & Tenen, 1966; Holtzmann & Jewett, 1971; Schulz & Frey, 1972; Kruk, 1973; Groppetti, Barzaghi & Mantegazza, 1973a; Groppetti, Zambotti, Biazzi & Mantegazza, 1973b; Leibowitz, 1975), while fenfluramine, although chemically related to (+)-amphetamine, induces anorexia via direct or indirect indoleamine-like effects (Funderburk, Hazelwood, Ruckart & Ward, 1971; Samanin, Ghezzi, Valzelli & Garattini, 1972; Clineschmidt, 1973; Jespersen & Scheel-Krüger, 1973).

Mazindol (5-hydroxy-5-*p*-chlorophenyl-2,3-dihydro-5H-imidazo-2,1-a-isoindole) (Figure 1), a drug with a different chemical structure from those of the β-phenylethylamines, was found in our laboratories to possess a behavioural profile similar to that of (+)-amphetamine (Zambotti, Carruba, Barzaghi, Vicentini, Groppetti & Mantegazza, 1975). In fact mazindol, when given to rats, in addition to causing anorexia, increased motor activity and body temperature, elicited stereotyped behaviour and caused turning in animals with unilateral nigro-striatal lesions. Thus, it seemed of interest to investigate whether mazindol and (+)-amphetamine share not only their common behavioural profile, but also similar biochemical mechanisms. To this purpose, experiments on biogenic amine levels in whole brain and in discrete brain areas have been performed. In addition, because of the results obtained from these preliminary experiments, we decided to study the action of behaviourally active doses of mazindol on the turnover rate of brain 5-hydroxytryptamine (5-HT), noradrenaline (NA) and striatal dopamine.

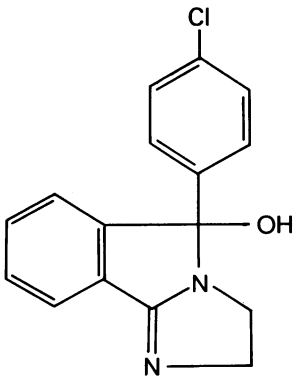

Figure 1 Chemical structure of mazindol.

Methods

All the experiments were performed on male Sprague-Dawley rats (Charles River) (150–200 g). Animals were killed by decapitation, the brain was removed, and in some instances the hypothalamus or caudate nucleus was dissected out, frozen on dry ice and stored at −20°C until assayed. Details of dose and time are given in the relevant places throughout the paper.

Determination of catecholamines

The tissues were homogenized in ice-cold 0.4 N perchloric acid containing 0.1% metabisulphite ($Na_2S_2O_5$) and centrifuged. The catecholamines in the supernatant fluid were absorbed onto Al_2O_3 and then extracted with 0.2 N acetic acid as described by Brodie, Comer, Costa & Dlabac (1966a) and assayed fluorimetrically according to the method of Chang (1964).

Determination of 5-hydroxyindoleacetic acid and 5-hydroxytryptamine

The brain was homogenized with 10 volumes of acidified butanol and centrifuged. The butanol phase was used for 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) determination as described by Curzon & Green (1970).

Measurement of dopamine turnover

Rats were injected intraperitoneally with 300 mg/kg of α-methyl-*p*-tyrosine methylester hydrochloride (AMPT) 15 min after mazindol (5 or 10 mg/kg, s.c.) or 0.9% w/v NaCl solution (saline) and killed 1.5 and 3 h thereafter. The caudate nucleus was then immediately dissected from the brain and stored frozen at −20°C until assayed. The frozen tissue samples were homogenized in 0.4 N perchloric acid and dopamine was assayed as previously described. The turnover rate of caudate dopamine was calculated from the product of the steady state of dopamine and the rate constant of dopamine decline (Brodie, Costa, Dlabac, Neff & Smookler, 1966b).

Measurement of noradrenaline turnover

[3,5-^{3}H]-L-tyrosine was diluted in saline so that an injection volume of 0.5 ml per 100 g body weight corresponded to 1.0 mCi/kg. Mazindol was injected subcutaneously 40 min before the intravenous injection of [^{3}H]-tyrosine. Animals were killed 20 min after the injection of the labelled compound. Brain samples were homogenized in 0.4 N perchloric acid containing 0.1% sodium metabisulphite. The homogenate was centrifuged and 4.7 ml of the supernatant fluid were removed and adjusted to pH 4.2 by addition of 0.3 ml of 10M potassium acetate. After a second centrifugation, 4 ml of the clear supernatant fluid was placed on a Dowex AG 50Wx4, 200–400 mesh, column (25 mm × 25 mm^2) prepared as described by Haggendal (1963). Elution of the columns and radiometric and fluorimetric assay of NA and tyrosine were done as described by Neff, Spano, Groppetti, Wang & Costa (1971). The conversion index (CI) of the [^{3}H]-tyrosine into brain NA was determined by dividing the radioactivity of the amine (d min^{-1} g^{-1}) by the specific activity (sp. act.) of tyrosine (Ty) (Costa *et al.*, 1971).

$$CI = \frac{\text{d min}^{-1}\,\text{g}^{-1}\,(\text{NA})}{\text{sp. act. (Ty)}}$$

Measurement of 5-hydroxytryptamine turnover

Brain 5-HT turnover rate was calculated, as described by Neff, Tozer & Brodie (1966), from the decline of brain 5-HIAA after intraperitoneal administration of

Table 1 Whole brain monoamine concentrations (noradrenaline (NA), dopamine (DA) and 5-hydroxytryptamine (5-HT)) in the rat after administration of different doses of mazindol

Treatment	Dose (mg/kg s.c.)	Time* (h)	NA	DA (μg/g ± s.e.)	5-HT
Saline	—	—	0.43 ± 0.007	1.08 ± 0.024	0.58 ± 0.017
Mazindol	2.5	1	0.47 ± 0.018	1.07 ± 0.040	0.59 ± 0.021
	5.0	1	0.45 ± 0.020	1.07 ± 0.012	0.55 ± 0.021
	10.0	1	0.42 ± 0.010	1.08 ± 0.022	0.59 ± 0.011
	5.0	3	0.40 ± 0.014	1.00 ± 0.020	0.56 ± 0.016
	2.5	5	0.45 ± 0.016	1.18 ± 0.043	0.62 ± 0.032
	5.0	5	0.47 ± 0.010	1.15 ± 0.053	0.56 ± 0.032
	10.0	5	0.41 ± 0.005	1.11 ± 0.052	0.57 ± 0.018

* Time after the drug administration when animals killed. Each value represents the mean ± s.e. of 10–20 rats.

90 mg/kg pargyline alone or 10 min after mazindol 5 mg/kg subcutaneously. Animals were killed 30 and 60 min after pargyline administration.

Statistics

Data were analyzed using Student's t test; the level of significance was chosen as $P=0.05$. The method of least squares was used to calculate the best-fit curves. Furthermore an analysis of variance has been performed in order to evaluate the significance of the regression and parallelism.

Drugs

Drugs were dissolved in 0.9% w/v NaCl solution (saline) unless otherwise stated. The injection volume was always 0.5 ml per 100 g body weight. For mazindol the doses refer to the base, for the other drugs to the salt. α-Methyl-L-*p*-tyrosine methylester hydrochloride and pargyline hydrochloride were generously supplied by Hoffman-La Roche, Research Division, Basel, and mazindol by Sandoz, Milan. [3,5-^{3}H]-L-Tyrosine (53 Ci/mmol) was obtained from the Radiochemical Centre, Amersham.

Results

Tissue concentrations of monoamines

The concentrations of NA, dopamine and 5-HT in brain tissue at different times after the subcutaneous injection of various doses of mazindol (2.5, 5 or 10 mg/kg, s.c.) are shown in Table 1. These data indicate that brain amine levels were not significantly different from those of the controls at any dose and any time studied. Similarly, administration of the same doses of mazindol did not alter the hypothalamic NA content when measured 1 h after drug treatment (Table 2). Table 3 shows that the concentrations of caudate dopamine were also unaffected after injection of mazindol, 2.5, 5 or 10 mg/kg subcutaneously over a 5 h period.

*Effect of mazindol on rate of caudate dopamine decline after α-methyl-*p*-tyrosine*

Table 4 shows the effect of mazindol on the decline of caudate dopamine concentrations elicited by AMPT. In saline-treated rats, after blockade of catecholamine synthesis with AMPT (300 mg/kg, i.p.), the caudate dopamine concentrations declined exponentially with a rate constant of 0.28 ± 0.027 (h^{-1}). Fifteen min after mazindol administration (5 or 10 mg/kg, s.c.) caudate dopamine levels were unaffected and the rate of the dopamine decline after AMPT was statistically higher than that of controls only when 10 mg/kg was injected (Table 4).

Effect of mazindol on turnover rate of brain noradrenaline

The effects of mazindol and saline on the CI of [^{3}H]-tyrosine into brain NA were compared. The results shown in Table 5 indicate that mazindol at a dose of 2.5 and 5 mg/kg subcutaneously did not change the concentrations and the sp. act. of brain tyrosine and did not alter steady state levels of brain NA of rats receiving the labelled compound 40 min after the drug. Mazindol at these doses decreased the CI of tyrosine into NA and the effect was statistically significant and dose-related.

Table 2 Hypothalamic noradrenaline (NA) concentrations in the rat after acute administration of different doses of mazindol

Treatment	*Dose (mg/kg s.c.)*	*NA * (μg/g ± s.e.)*
Saline	—	2.57 ± 0.06
Mazindol	2.5	2.37 ± 0.15
	5.0	2.68 ± 0.10
	10.0	2.46 ± 0.16

* One hour after treatment. Each value represents the mean ± s.e. of 5 animals.

Table 3 Effect of mazindol on caudate dopamine concentrations in the rat.

Dose of mazindol (mg/kg s.c.)	*Dopamine (hours after treatment)* 1	3	5
2.5	110 ± 4.5	—	89 ± 2.8
5.0	103 ± 6.6	108 ± 2.9	99 ± 6.3
10.0	107 ± 2.1	—	100 ± 4.9

The figures indicate mean values ± s.e. for 8 rats and are expressed as % of control values. Control values: dopamine 10.28 ± 0.44 μg/g.

Effect of mazindol on 5-hydroxyindoleacetic acid rate of decline after pargyline

The rate of decline of brain 5-HIAA after treatment with pargyline (90 mg/kg, i.p.), as is shown in Table 6, could not be altered by administration of mazindol, (5 mg/kg, s.c.). Calculation of turnover rate constants gave a value of $K = 0.75 \pm 0.03$ (h^{-1}) for saline-treated controls that was not statistically different from that obtained in mazindol-pretreated animals ($K = 0.84 \pm 0.02$ h^{-1}).

Discussion

The brain biogenic amines (NA, dopamine, 5-HT) levels were measured in rats receiving behaviourally active doses of mazindol over a 5 h time period. None of the doses injected caused a change of these amine concentrations at any time studied. No changes were detected even when NA and dopamine levels were measured in the hypothalamus and in the caudate nucleus respectively. Thus, these results indicate that mazindol is not similar to (+)-

Table 4 Turnover rates and turnover times of caudate dopamine, measured from dopamine decline, after blockade of synthesis by α-methyl-*p*-tyrosine (AMPT) (300 mg/kg i.p.)

Treatment	*Steady-state levels (μg/g ± s.e.)*	*Rate constant of amine loss (K(h^{-1}) ± s.e.)*	*Turnover time† (h)*	*Turnover rate† (μg g^{-1} h^{-1})*
Controls	9.25 ± 0.30	0.28 ± 0.027	3.5	2.64
Mazindol‡ (5 mg/kg s.c.)	10.02 ± 0.26	0.37 ± 0.030	2.6	3.73
Controls	11.63 ± 0.43	0.29 ± 0.032	3.4	3.39
Mazindol‡ (10 mg/kg s.c.)	12.02 ± 0.34	0.55 ± 0.035*	1.8	6.61

* $P < 0.01$.
† Turnover rate is the product of the steady state level and K, the rate constant of dopamine decline; turnover time is $1/K$. ‡ Mazindol was given 15 min before AMPT treatment.

Table 5 Effect of mazindol on conversion of [3,5-^{3}H]-L-tyrosine into brain noradrenaline (NA)

Treatment	*Dose (mg/kg s.c.)*	*No. of rats*	*Tyrosine ($d\ min^{-1}\ nmol^{-1}$ ± s.e. × 10^{-3})*	*NA (nmol/g ± s.e.)*	*Conversion index (NA nmol g^{-1} 20 min^{-1} ± s.e.)*
Saline	—	7	5.24 ± 0.28	2.40 ± 0.13	0.56 ± 0.06
Mazindol	2.5	8	5.97 ± 0.23	2.49 ± 0.11	0.38 ± 0.02*
Saline	—	7	3.59 ± 0.08	1.95 ± 0.08	0.49 ± 0.03
Mazindol	5.0	6	4.22 ± 0.31	2.04 ± 0.08	0.22 ± 0.01†

* $P < 0.02$; † $P < 0.001$.

Table 6 Turnover rate of brain 5-hydroxytryptamine (5-HT) and turnover time of 5-hydroxyindoleacetic acid (5-HIAA) as measured from the decline of 5-hydroxyindoleacetic acid after pargyline

Treatment	*Steady-state brain level of 5-HIAA (μg/g ± s.e.)*	*Rate constant of 5-HIAA loss after pargyline (K(h^{-1}) ± s.e.)*	*Turnover time of 5-HIAA (h)*	*Turnover rate of 5-HT (μg g^{-1} h^{-1})*
Controls	0.662 ± 0.04	0.754 ± 0.036	1.32	0.4994
Mazindol (5 mg/kg s.c.)	0.717 ± 0.03	0.845 ± 0.025	1.18	0.6063

amphetamine or fenfluramine since (+)-amphetamine decreases tissue concentrations of NA (McLean & McCartney, 1961; Moore & Lariviere, 1963; Carr & Moore, 1969; Groppetti & Costa, 1969; Costa, Groppetti & Revuelta, 1971) while fenfluramine reduces both 5-HT and NA concentrations of brain tissue (Duce & Gessa, 1966; Opitz, 1967; Duhault & Verdavainne, 1967; Costa *et al.*, 1971).

Since changes in the turnover rates of neurohormones are believed to be a better index of neuronal activity than changes in the concentrations of the amines, which might remain virtually unchanged despite marked changes in their rates of synthesis, turnover studies of brain NA, 5-HT and striatal dopamine were performed. The data obtained clearly show that mazindol, like (+)-amphetamine (Groppetti, Misher, Naimzada, Revuelta & Costa, 1972; Groppetti *et al.*, 1973b) increases the dopamine turnover rate in the caudate nucleus. However, in contrast to (+)-amphetamine and fenfluramine (Groppetti *et al.*, 1972; Groppetti *et al.*, 1973b) mazindol decreases the incorporation rate of [^{3}H]-tyrosine into brain NA in a dose-related manner. Furthermore, in our experimental conditions, mazindol did not modify the rate constant of 5-HIAA decline following pargyline administration. In a previous report (Zambotti *et al.*, 1975), on the basis of the observation that the anorectic effect of mazindol can be antagonized by pretreatment with AMPT, an inhibitor of catecholamine synthesis (Spector, Sjoerdsma & Udenfriend, 1965), and with pimozide, a selective dopamine blocker (Andén, Butcher, Corrodi, 1970), we pointed out that a presynaptic dopaminergic mechanism resulting in increased dopamine release might be involved in mazindol-induced anorexia. Further support for the suggestion that mazindol causes dopamine release comes from the finding that rats with unilateral lesions in the substantia nigra began to rotate to the side of the lesion (behaviour related to dopamine release in the striatum of the non-lesioned side) after the administration of mazindol (Zambotti *et al.*, 1975). The present data on dopamine turnover also confirm this hypothesis on a biochemical basis and are consistent with an observation of Jori & Dolfini (1974) who found an increase in the brain homovanillic acid concentration after 15 mg/kg of mazindol. Thus, we might conclude that the mechanisms whereby mazindol induces anorexia and turning behaviour depend upon an increased synthesis and release of dopamine from its nerve terminals. Among the several possibilities for the effects of mazindol on NA, the one that appears most likely is that the deceleration of NA turnover by mazindol could be the result of an increased receptor stimulation which, through a negative feed-back mechanism, inhibits NA biosynthesis in the presynaptic neurones. It has been recently reported, in fact, by Engstrom, Kelly & Gogerty (1975) that mazindol is able to block the neuronal uptake of NA, as shown by the reduction in uptake of intraventricularly injected [^{3}H]-NA.

The failure to detect any alteration of the pargyline-induced decline of brain 5-HIAA after mazindol treatment can be taken as evidence that this drug does not modify 5-HT turnover. Based on this finding, it seems possible to rule out for mazindol-induced anorexia, the same mechanism of action suggested for fenfluramine (Funderburk *et al.*, 1971; Samanin *et al.*, 1972; Jespersen & Scheel-Krüger, 1973; Clineschmidt, 1973). In conclusion, mazindol, although having a behavioural profile similar to that of (+)-amphetamine (Zambotti *et al.*, 1975) shows some differences in its neurochemical effects. Moreover the data reported here show that mazindol does not share any biochemical effects with fenfluramine.

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References

ANDÉN, N.E., BUTCHER, S.G., CORRODI, M., FUXE, K. & UNGERSTEDT, U. (1970). Receptor activity and turnover of dopamine and noradrenaline after neuroleptics. *Eur. J. Pharmac.*, **11**, 303–314.

BRODIE, B.B., COMER, M.S., COSTA, E. & DLABAC, A. (1966a). The role of brain serotonin in the mechanism of the central action of reserpine. *J. Pharmac. exp. Ther.*, **152**, 340–349.

BRODIE, B.B., COSTA, E., DLABAC, A., NEFF, N.H. & SMOOKLER, H.H. (1966b). Application of steady state kinetics to the estimation of synthesis rate and turnover time of tissue catecholamines. *J. Pharmac. exp. Ther.*, **154**, 493–498.

CARR, L.A. & MOORE, K.E. (1969). Norepinephrine: release from brain by d-amphetamine *in vivo*. *Science*, **164**, 322–323.

CHANG, C.C. (1964). A sensitive method for spectrophotofluorimetric assay of catecholamines. *Int. J. Neuropharmac.*, **3**, 643–649.

CLINESCHMIDT, B.V. (1973). 5,6-Dihydroxytryptamine: suppression of the anorexigenic action of fenfluramine. *Eur. J. Pharmac.*, **24**, 405–409.

COSTA, E., GROPPETTI, A., REVUELTA, A. (1971). Action of fenfluramine on monoamine stores of rat tissues. *Br. J. Pharmac.*, **41**, 57–64.

CURZON, G. & GREEN, A.R. (1970). Rapid method for the determination of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in small regions of the rat brain. *Br. J. Pharmac.*, **39**, 653–655.

DUCE, M. & GESSA, G.L. (1966). Deplezione di catecolamine centrali e periferiche indotta da Fenfluramina. *Boll. Soc. It. Biol. Sper.*, **42**, 1631–1637.

DUHAULT, J. & VERDAVAINNE, C. (1967). Modification du taux de serotonine cérebrale chez le rat par les trifluorométhyl-phényl-2-ethylamino propane (Fenfluramine 7685). *Arch. Int. Pharmacodyn. Thér.*, **170**, 276–286.

ENGSTROM, R.G., KELLY, L.A. & GOGERTY, J.H. (1975). The effects of 5-hydroxy-5-(4-chlorophenyl)-2,3-Dihydro-5H-imidazo (2,I-a) isoindole (Mazindol, 5aH 42-548) on the metabolism of brain norepinephrine. *Arch. Int. Pharmacodyn.*, **214**, 308–321.

FUNDERBURK, W.H., HAZELWOOD, J.C., RUCKART, R.T. & WARD, J.W. (1971). Is 5-hydroxytryptamine involved in the mechanism of action of fenfluramine? *J. Pharm. Pharmac.*, **23**, 468–469.

GROPPETTI, A. & COSTA, E. (1969). Tissue concentrations of p-hydroxynorephedrine (pOHNE) injected with d-amphetamine: Effect of pretreatment with desipramine (DMI). *Life Sci.*, **8**, 653–665.

GROPPETTI, A., MISHER, A., NAIMZADA, M., REVUELTA, A. & COSTA, E. (1972). Evidence that in rats I-Benzyl-β-Metoxy-3-trifluoromethylphenethylamine (SK&F I-39728) dissociates anorexia from central stimulation and action on brain monoamine stores. *J. Pharmac. exp. Ther.*, **182**, 464–473.

GROPPETTI, A., BARZAGHI, F. & MANTEGAZZA, P. (1973a). Ruolo delle catecolamine nell'anoressia da amfetamina. *Riv. Farmacol. Terap.*, **4**, 111a–120a.

GROPPETTI, A., ZAMBOTTI, F., BIAZZI, A. & MANTEGAZZA, P. (1973b). Amphetamine and cocaine on amine turnover. In *Frontiers in Catecholamine Research*, ed. Usdin, E. & Snyder, S.H., pp. 917–925. Oxford: Pergamon Press.

HAGGENDAL, J. (1963). An improved method for fluorimetric determination of small amounts of adrenaline and noradrenaline in plasma and tissues. *Acta physiol. scand.*, **59**, 242–254.

HOLTZMAN, S.G. & JEWETT, R.E. (1971). The role of brain norepinephrine in the anorexic effect of dextro-amphetamine and monoamine oxidase inhibitors in the rat. *Psychopharmacologia*, **22**, 151–161.

JESPERSEN, S. & SCHEEL-KRÜGER, J. (1973). Evidence for a difference in mechanism of action between fenfluramine and amphetamine-induced anorexia. *J. Pharm. Pharmac.*, **25**, 49–54.

JORI, A. & DOLFINI, E. (1974). On the effect of anorectic drugs on striatum Homovanillic acid in rats. *Pharmac. Res. Comm.*, **6**, 175–178.

KRUK, Z.L. (1973). Dopamine and 5-hydroxytryptamine inhibit feeding in rats. *Nature New Biol.*, **246**, 52–53.

LEIBOWITZ, S.F. (1975). Amphetamine: possible site and mode of action for producing anorexia in the rat. *Brain Res.*, **84**, 160–167.

McLEAN, J.R. & McCARTNEY, M. (1961). Effect of d-amphetamine on rat brain noradrenaline and serotonin. *Proc. Soc. exp. Biol. Med.*, **107**, 77–79.

MOORE, K.E. & LARIVIERE, E.W. (1963). Effects of d-amphetamine and restraint on the content of norepinephrine and dopamine in rat brain. *Bioch. Pharmac.*, **12**, 1283–1288.

NEFF, N.H., TOZER, T.N., BRODIE, B.B. (1966). Application of steady state kinetics to the synthesis rate and turnover time of serotonin in the brain of normal and reserpine-treated rats. *J. Pharmac. exp. Ther.*, **153**, 177–182.

NEFF, N.H., SPANO, P.F., GROPPETTI, A., WANG, C.T. & COSTA, E. (1971). A simple procedure for calculating the synthesis rate of norepinephrine, dopamine and serotonin in rat brain. *J. Pharmac. exp. Ther.*, **176**, 701–710.

OPITZ, K. (1967). Anorexigene phenylalkylamine und Serotoninstoffwechsel. *Naunyn-Schmiedebergs Arch. Pharmak. exp. Path.*, **259**, 56–65.

SAMANIN, R., GHEZZI, D., VALZELLI, L. & GARATTINI, S. (1972). The effects of selective lesioning of brain serotonin or catecholamine containing neurones on the anorectic activity of fenfluramine and amphetamine. *Eur. J. Pharmac.*, **19**, 318–322.

SCHULZ, R. & FREY, H.H. (1972). Study into the mechanism of the anorectic action of amphetamine and its p-chloro analogue. *Acta pharmac. et toxicol.*, **31**, suppl. 1, 12.

SPECTOR, S., SJOERDSMA, A. & UDENFRIEND, S (1965). Blockade of endogenous norepinephrine synthesis by α-methyl-tyrosine, an inhibitor of tyrosine hydroxylase. *J. Pharmac. exp. Ther.*, **147**, 86–95.

WEISSMAN, A., KOE, B.K. & TENEN, S.S. (1966). Antiamphetamine effects following inhibition of tyrosine hydroxylase. *J. Pharmac. exp. Ther.*, **151**, 339–352.

ZAMBOTTI, F., CARRUBA, M.O., BARZAGHI, F., VICENTINI, L., GROPPETTI, A. & MANTEGAZZA, P. (1975). Behavioural effects of a new nonphenyl-ethylamine anorexigenic agent: Mazindol. *Eur. J. Pharmac.*, (in press).

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