Br. J. Pharmac. (1978), **62**, 579–589

A 5-HYDROXYTRYPTAMINE-LIKE MODE OF ANORECTIC ACTION FOR 6-CHLORO-2-[1-PIPERAZINYL]-PYRAZINE (MK-212)

BRADLEY V. CLINESCHMIDT, JODIE C. McGUFFIN, A. BARBARA PFLUEGER & JAMES A. TOTARO

Merck Institute for Therapeutic Research, West Point, Pa. 19486, U.S.A.

1 The mechanism of the reduction in food consumption elicited by 6-chloro-2-[1-piperazinyl]-pyrazine (MK-212) administered systemically was investigated in the rat. (±)-Fenfluramine and (+)-amphetamine were included in some studies for comparative purposes.

2 Pretreatment with methergoline, a 5-hydroxytryptamine (5-HT) antagonist, reduced the magnitude of the anorectic effect of 1.5 and 3 mg/kg of MK-212, while the anti-5-HT agents, cyproheptadine and cinanserin, were likewise effective against the 3 mg/kg dose.

3 Xylamidine, an antagonist of 5-HT that penetrates poorly into the central nervous system, completely blocked the decrease in food intake caused by 5-HT administered peripherally, while not antagonizing an equianorectic dose of MK-212.

4 Reduction of brain 5-HT by intraventricular injection of 5,6-dihydroxytryptamine, intraperitoneal administration of *p*-chloroamphetamine or placement of a lesion in the region of the median raphé nucleus diminished the anorectic response to 3 mg/kg of MK-212. The anorectic effect of amphetamine was reduced by *p*-chloroamphetamine or lesion in the raphé, but not by 5,6-dihydroxytryptamine. The decrease in food consumption produced by 1.5 mg/kg of MK-212 was antagonized by prior treatment with *p*-chloroamphetamine, but not by 5,6-dihydroxytryptamine.

5 Haloperidol, which blocks receptors for dopamine, antagonized the anorexigenic effect of amphetamine, but was ineffective in offsetting the action of MK-212, 3 mg/kg.

6 Pretreatment with chlorimipramine to inhibit the 5-hydroxytryptaminergic uptake mechanism did not affect the anorectic response to 3 mg/kg of MK-212, whereas the response to fenfluramine was diminished.

7 The results indicate that the anorectic action of MK-212 involves a 5-HT-like component which is more evident at the higher dose level of the compound. The anorexigenic property of MK-212 may depend, at least partly, upon the integrity of 5-HT-containing neurones in the central nervous system.

Introduction

The anorexigenic and ancillary properties in animals of 6-chloro-2-(1-piperazinyl)pyrazine (MK-212) were recently described (Clineschmidt, Hanson, Pflueger & McGuffin, 1977). Unlike amphetamine and related substances, MK-212 greatly reduced food consumption without an accompanying general stimulation of the central nervous system. Locomotor activity in rodents was inconsistently and only slightly affected by MK-212, and cats appeared mildly sedated after anorectic doses of the compound. At least superficially, MK-212 resembles fenfluramine, since both cause anorexia without evoking overt signs of central nervous system stimulation. Fenfluramine is a derivative of amphetamine, but, in addition to causing moderate sedation in both animals and man (Pinder, Brogden, Sawyer, Speight & Avery, 1975), recent studies in animals indicate that fenfluramine also differs significantly from the parent compound with respect to its primary mode of anorectic action. While the release of catecholamines, especially dopamine, from central monoamine-containing neurones is an important aspect of amphetamine's action (Weissman, Koe & Tenen, 1966; Abdalah, 1971; Holtzman & Jewett, 1971; Schulz & Frey, 1972; Clineschmidt, McGuffin & Werner, 1974; Hollister, Ervin, Cooper & Breese, 1975), fenfluramine acts mainly via a direct or indirect 5-hydroxytryptamine (5-HT)-mimetic effect (Funderburk, Hazelwood, Ruckart & Ward,

1971; Samanin, Ghezzi, Valzelli & Garattini, 1972; Jespersen & Scheel-Kruger, 1973; Clineschmidt, 1973; Clineschmidt *et al.*, 1974; Fuxe, Farnebo, Hamberger & Ogren, 1975b; Garattini, Buczko, Jori & Samanin, 1975).

The objective of the present investigation was to determine whether MK-212 produces an anorectic response in the rat through a 5-HT-like action, thereby resembling fenfluramine rather than amphetamine. Evidence will be give for a 5-HT-like component in the anorectic action of MK-212, for the central nervous system as the site of this effect and for the possible involvement of 5-hydroxytryptaminergic neurones in MK-212-induced anorexia.

Methods

Female CFE Carworth rats weighing 180 to 230 g were trained to eat from special jars (Joy, Emma & Mayer, 1967) designed to minimize spillage of the food (powdered Purina Chow). The animals were maintained in individual cages and allowed to eat for only 2 h per day (either 09 h 00 min to 11 h 00 min or 13 h 00 min to 15 h 00 min). During the period (1–3 weeks) of adjustment to the feeding jars and schedule, those rats that regularly spilled food onto the floor of their cages were removed from the test colony. The general protocol was to compare the amount of food eaten on the test (drug) day with the amount consumed on the immediately preceding (control) day. For each animal, the amount of food consumed on the test day was expressed as a percentage of the control day. On the test day, rats were pretreated intraperitoneally with vehicle or various drugs before intraperitoneal injection with MK-212, fenfluramine or amphetamine. The doses of the compounds used for pretreatment were selected on the basis of previous findings, establishing effectiveness and selectivity with respect to reducing the anorectic activity of either amphetamine or fenfluramine (Clineschmidt *et al.*, 1974). Treatment with one of the anorexigens was usually at twice the dose required to reduce food intake by 50% (ED_{50}). Some studies were also performed using the ED_{50} level of the anorectic compounds. The ED_{50} values for MK-212, fenfluramine and amphetamine were determined in preliminary experiments by regression analysis of log dose and % decrease in food consumption. ED_{50} values were, respectively, 1.5, 1.5 and 3 mg/kg for MK-212, amphetamine and fenfluramine. Haloperidol was injected 2.5 h before treatment, and methergoline, cyproheptadine, cinanserin and chlorimipramine were administered 30 min before treatment. Thirty min after treatment with MK-212, fenfluramine or amphetamine, the test meal was presented to the rats. The basic conditions listed above were changed somewhat for a study on the effect of pretreatment with xylamidine on the anorectic responses to 5-HT and MK-212. The rats were allowed to eat for 3 h per day rather than the usual 2 h, and treatment was given subcutaneously 15 min before the test meal was given to the rats rather than treating the animals intraperitoneally 30 min before feeding.

Female CFE Carworth rats weighing about 180 g were anaesthetized with ether. Using the method devised by Noble, Wurtman & Axelrod (1967), 5,6-dihydroxytryptamine (75 μg of the base dissolved in sterile water for injection containing 0.1 mg ascorbic acid per ml) or ascorbate vehicle was injected into the right lateral ventricle (injection volume of 10 μl). In preliminary experiments, we found that accurate injection of 5,6-dihydroxytryptamine into the ventricle resulted in marked anorexia (< 3 g consumed) the following day. Therefore, rats accustomed to the 22 h food deprivation schedule were injected intraventricularly and then selected for subsequent testing with MK-212 or amphetamine based on this anorectic response. After ascertaining food intake on the day after administering 5,6-dihydroxytryptamine, the animals were allowed to eat *ad libitum* before being placed back on the 22 h deprivation schedule one week later. The rats were treated intraperitoneally 30 min before feeding on the 14th day after the intraventricular injection. Twenty four h after treatment with MK-212 or amphetamine, the animals' whole brains were removed and frozen on Dry Ice for subsequent assay.

Female Carworth rats weighing approximately 180 g and maintained on the usual 22 h food deprivation schedule were injected intraperitoneally with *p*-chloroamphetamine, 15 mg/kg, or saline 1 h after removal of food on Day 0. Food consumption was ascertained on Day 8 (control day) and again on Day 9 (test day). Intraperitoneal injections of MK-212, fenfluramine or amphetamine were given 30 min before presentation of food on the test day. A group of rats not used for food consumption studies was injected intraperitoneally with *p*-chloroamphetamine, 15 mg/kg, or 0.9% w/v NaCl solution (saline) 216 h (9 days) before they were killed by decapitation. The whole brain was frozen on Dry Ice and stored at about $-5°C$ until assayed for content of monoamines.

Female Charles River (CRCD COBS) rats weighing 160 to 180 g were anaesthetized with hexobarbitone (75 mg/kg i.p.), and, with the aid of a David Kopf (Tujunga, California) stereotaxic instrument, the atlas of König & Klippel (1963) and a Grass Instruments (Quincy, Massachusetts) constant current lesion maker (Model DCLM 5), a lesion was placed in the region of the median raphé nucleus. The coordinates were frontal +0.4, saggital 0 and horizontal −2.9 mm. The electrode (stainless steel coated with Epoxylite, exposed for a length of 1 mm at the tip and

having a diameter of 0.35 mm at the tip) was inserted at an angle of 30°. Anodal current of 2.5 mA was passed for 15 seconds. Sham controls were treated identically, except that current was not passed through the electrode. Nine to 10 days after the operation, the animals were individually caged and subjected to the 22 h food deprivation schedule. Nineteen to 21 days following the operation, an injection of MK-212, fenfluramine or amphetamine was given 30 min before presentation of food. Forty-eight h after injection of drug, the animals were decapitated and their brains were removed. The brain was divided by a frontal section running anterior to the inferior colliculus and pons. The forebrain was immediately frozen on Dry Ice for subsequent assay of monoamines. The caudal portion of the brain was placed in 37% formalin for 3 days and then examined macroscopically with the aid of a cryostat for the location of the lesion. While the results were excluded when the lesion was clearly not in the region of the median raphé nucleus, the level of 5-HT in the forebrain was also considered. Data obtained from a particular lesioned rat and its matched sham control were excluded from the study if the concentration of 5-HT in the forebrain of the lesioned animal was reduced by less than 20% compared with the mean concentration in the forebrains from sham controls that were assayed concurrently.

The whole brain or forebrain was ground with 10 volumes of 0.4 N perchloric acid, and the resultant homogenate was centrifuged at 15,000 *g* for 15 minutes. One 4 to 5 ml aliquot of the clear perchloric acid supernatant solution was taken for estimation of catecholamines and another for determination of 5-HT. Catecholamines were adsorbed from the perchloric acid tissue extracts on alumina (Anton & Sayre, 1962) and eluted with 0.05 N HCl, then assayed fluorometrically (Porter, Totaro & Burcin, 1965). The aliquot used for determination of 5-HT was added to 5 ml of 0.5 M borate buffer, pH 10 (not saturated with NaCl or butanol), and sufficient 1 N NaOH was added to adjust the sample to pH 10. The remainder of the procedure was performed according to Bogdanski, Pletscher, Brodie & Udenfriend (1956).

The doses were adjusted to account for any weight contributed by the presence of a salt. The volume of injection for both pretreatment and treatment was 0.1 ml/100 g of body weight. MK-212 hydrochloride (synthesized by Dr William C. Lumma, Medicinal Chemistry Department, Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania), (±)-fenfluramine hydrochloride (A. H. Robins, Richmond, Virginia), (+)-amphetamine sulphate (Merck Sharp & Dohme Research Laboratories) and 5-hydroxytryptamine (serotonin) creatinine sulphate (Aldrich Chemical Company, Milwaukee, Wisconsin) were dissolved in distilled water. (±)-*p*-Chloroamphetamine hydrochloride (Regis Chemical Company, Morton Grove, Illinois) was dissolved in saline. Xylamidine tosylate (The Wellcome Research Laboratories, Beckenham, England), cinanserin hydrochloride (E. R. Squibb & Sons, Princeton, New Jersey), cyproheptadine hydrochloride (Merck Sharp & Dohme Research Laboratories), methergoline (Farmitalia, Milan, Italy), chlorimipramine hydrochloride (Ciba-Geigy Pharmaceuticals, Ardsley, New York) and haloperidol (McNeil Laboratories, Fort Washington, Pennsylvania) were suspended/dissolved in 1% methylcellulose.

The *t* test (two-tailed) was employed for analysis of the significance of observed differences among treatment groups. A P value of <0.05 was taken as significant.

Results

Reduction of the anorectic effect of MK-212 by antagonists of 5-hydroxytryptamine

Pretreatment with methergoline (Beretta, Glasser, Nobili & Silvestri, 1965; Ferrini & Glasser, 1965; Clineschmidt & Lotti, 1974) significantly reduced the magnitude of the anorectic response to MK-212, 1.5 (approximate ED_{50}) and 3 mg/kg (Figure 1). Methergoline alone did not affect food consumption. The anorexigenic action of MK-212, 3 mg/kg, was also antagonized by prior treatment with cyproheptadine (Stone, Wenger, Ludden, Stavorski & Ross, 1961) or cinanserin (Rubin, Piala, Burke & Craver, 1964), as shown in Figure 2. Cyproheptadine itself caused a small but significant reduction in the amount of food eaten, whereas food intake was not affected by cinanserin alone. Cyproheptadine and cinanserin were not effective as antagonists of MK-212-induced anorexia when the anorexigen was given at a dose of 1.5 mg/kg (data not shown).

Xylamidine, an antagonist of 5-HT that penetrates only poorly into the central nervous system (Copp, Green, Hodson, Randall & Sim, 1967; Mawson & Whittington, 1970), completely blocked the anorectic response to 5-HT administered systemically (Figure 3). Xylamidine itself decreased food consumption. There was no difference ($P > 0.20$) between the xylamidine plus vehicle and xylamidine plus 5-HT groups with respect to the amount of food consumed. The overt manifestations of injected 5-HT, e.g. defaecation, ptosis, prostration, cyanosis, were also prevented by pretreatment with xylamidine. The reduction in the amount of food eaten caused by MK-212 was not antagonized by xylamidine (Figure 3). In fact, the group receiving xylamidine plus MK-212 had a lower ($P < 0.01$) consumption of food than the vehicle plus MK-212-treated animals. The individual decreases in

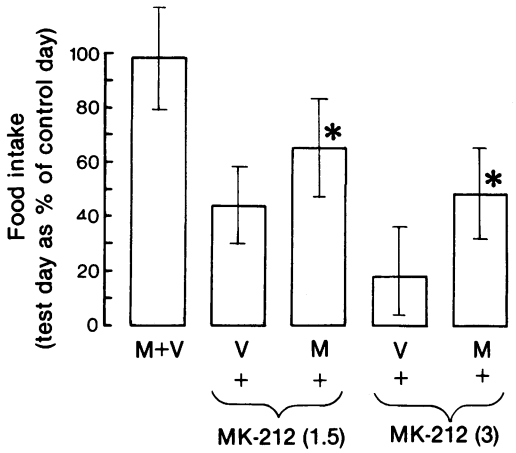

Figure 1 Antagonism by methergoline of MK-212- induced reduction in food intake. Methergoline (M, 1 mg/kg i.p.) was injected 30 min before MK-212, (1.5 or 3 mg/kg i.p.) and food was presented 30 min after the second injection. V = vehicle. Food consumption (g/2 h) was determined on two consecutive days, and, for each rat, the amount eaten on the test (drug) day was expressed as a percentage of that on the immediately preceding control day. The height of each column indicates the mean with the vertical line showing the s.d. of 6 animals per treatment group. *Indicates a statistically significant difference between pretreatment with vehicle and methergoline.

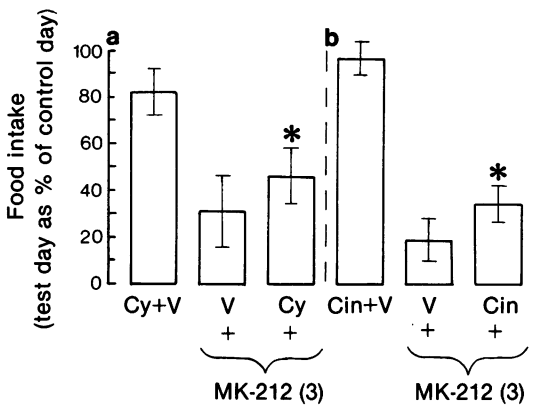

Figure 2 Antagonism of the anorectic activity of MK-212 by pretreatment with cyproheptadine (Cy) or cinanserin (Cin); (a) and (b) represent the results from separate experiments, i.e. the studies were not conducted concurrently. Cyproheptadine (1 mg/kg i.p.), and cinanserin (20 mg/kg i.p.) were administered 30 min before MK-212 (3 mg/kg i.p.). Food was presented to the rats 30 min after giving MK-212. V = vehicle. (a) Mean of 15 animals per group. (b) Mean of 6 rats/treatment group. Vertical lines show s.d. *Indicates that the MK-212-treated group pretreated with cyproheptadine or cinanserin differs significantly from corresponding animals pretreated with vehicle. Other details are as in Figure 1.

food intake resulting from xylamidine and MK-212 would appear to be additive.

Anorectic effect of MK-212 after chlorimipramine

Pretreatment with chlorimipramine did not affect the anorectic action of MK-212, whereas there was a diminution in the response to fenfluramine (Table 1).

Anorectic response to MK-212 after haloperidol

In rats previously treated with haloperidol, the anorectic effect of amphetamine was greatly reduced, but the ability of MK-212 to decrease food intake was unaffected (Table 2).

Effect of 5,6-dihydroxytryptamine on MK-212-induced anorexia

The anorectic response to 3 mg/kg of MK-212 was less in rats given an intraventricular injection of 5,6-dihydroxytryptamine 14 days earlier than in ascorbate-pretreated control animals (Table 3). The effect of MK-212 at 1.5 mg/kg was also reduced somewhat by prior 5,6-dihydroxytryptamine, but this was not statistically significant. In a comparable study with amphetamine, the compound was inadvertently administered at a dose of 2.5 mg/kg instead of the intended 3 mg/kg (twice the approximate ED_{50}). The magnitude of the decrease in food consumption caused by amphetamine was not affected by the previous administration of 5,6-dihydroxytryptamine. As shown in Table 3, the intraventricular injection of 5,6-dihydroxytryptamine caused a 32 to 44% reduction in whole brain 5-HT, while the concentrations of noradrenaline and dopamine were not consistently changed. The increase in noradrenaline of 18% in the 5,6-dihydroxytryptamine-treated group tested with amphetamine is regarded as a spurious finding, apparently due to the lower than usual concentration of the amine in brains from the concurrently assayed vehicle-treated control animals.

Anorectic activity of MK-212 after p*-chloroamphetamine*

Nine days after *p*-chloroamphetamine, brain 5-HT was reduced by 60%, noradrenaline was unchanged and dopamine was slightly decreased (6%) (Figure 4). On the day before testing for drug-induced anorexia

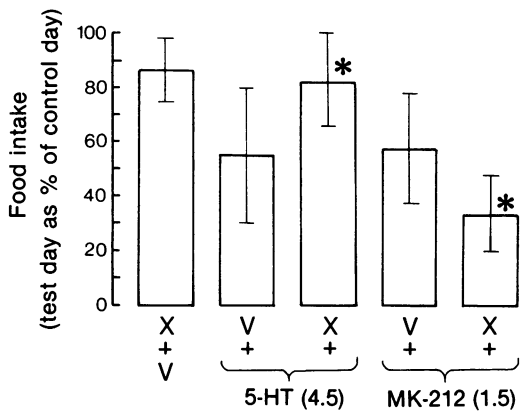

Figure 3 Effect of prior treatment with xylamidine (X) on the decrease in food consumption produced by 5-hydroxytryptamine (5-HT) and MK-212. The rats were pretreated with xylamidine (1 mg/kg i.p.) 30 min before treatment with 5-HT (4.5 mg/kg s.c.) or MK-212 (1.5 mg/kg s.c.). V = vehicle. Food was presented to the animals 15 min after the second injection. The data are the mean of 10–20 animals per group. Vertical lines show s.d. *Indicates that pretreatment with xylamidine differs significantly from corresponding group pretreated with vehicle. Other details as in Figure 1.

(i.e. 8 days after injection of *p*-chloroamphetamine), the consumption of food was sometimes lower in the *p*-chloroamphetamine-pretreated rats compared to vehicle-pretreated controls (Table 4). This was true in both studies with MK–212 as well as the experiment with the high dose level of amphetamine. The rats pretreated with *p*-chloroamphetamine and given 1.5 mg/kg of MK-212 ate on the average nearly 2 g more than the corresponding control animals, but the difference was not quite significant. Expressing food intake on the test day as a percentage of the preceding control day, there was, however, a significant difference in the response of the two different pretreatment groups to MK-212, 1.5 mg/kg (Table 4). The anorectic response to MK-212, 3 mg/kg, fenfluramine, 3 and 6 mg/kg, and amphetamine, 3 mg/kg, was reduced by prior administration of *p*-chloroamphetamine. *p*-Chloroamphetamine did not affect the anorectic activity of 1.5 mg/kg of amphetamine.

Effect of a lesion in the median raphé nucleus on MK-212-elicited anorexia

The anorectic activity of MK-212, 3 mg/kg, was less in animals with a lesion in the region of the median raphé nucleus (Table 5). Forebrains removed from these rats had a 45% reduction in 5-HT, while noradrenaline and dopamine were unchanged. Similar results were obtained with fenfluramine and amphetamine examined at twice their approximate ED_{50} for reducing food consumption (Table 5). It should be noted that forebrains from the group tested with amphetamine had a significant decrease in noradrenaline, unlike forebrains from the other two groups of lesioned animals. The greater response to fenfluramine in the sham-lesioned controls than observed previously in other control groups (Tables 1 and 4) is probably attributable to a strain difference. Rats from Charles River were used in the lesion studies, whereas animals from Carworth were employed for all other experiments. We found a higher post-lesion survival rate with the former strain, making it preferable for this particular part of the investigations.

Table 1 Effect of pretreatment with chlorimipramine on the anorexigenic actions of fenfluramine and MK-212

Pretreatment[a] *(mg/kg i.p.)*	*Treatment*[b] *(mg/kg i.p.)*	*Food intake*[c] *test day as % of preceding day*
Chlorimipramine (5)	Vehicle	84.0 ± 9.1
Vehicle	Fenfluramine (6)	11.0 ± 15.9
Chlorimipramine (5)	Fenfluramine (6)	40.5 ± 8.9*
Vehicle	MK-212 (3)	24.0 ± 11.0
Chlorimipramine (5)	MK-212 (3)	28.8 ± 12.7

[a] 30 min before treatment; [b] 30 min before presentation of food. [c] Food consumption was ascertained for 2 consecutive days; food intake on the test (drug) day is expressed as a percentage of that eaten on the immediately preceding (control) day. All values are mean ± s.d. of 6 rats per treatment.
* Significant difference compared to group pretreated with vehicle.

Discussion

The anorectic actions of MK-212 and amphetamine in the rat are easily differentiated by the use of pharmacological antagonists. Haloperidol, which blocks receptors for dopamine, reduces the response to amphetamine, but not MK-212, while anti-5-HT agents antagonize MK-212, but not amphetamine (present results and Clineschmidt *et al.*, 1974). Antagonists of 5-HT also reduce the magnitude of the anorectic response to fenfluramine, whereas haloperidol and other substances interrupting catecholamine-mediated neurotransmission are ineffective (Jespersen & Scheel-Kruger, 1973; Clineschmidt *et al.*, 1974; Hollister *et al.*, 1975). From these observations, MK-212 can be designated broadly as a fenfluramine-like rather than amphetamine-like anorexigen. A striking similarity does in fact exist between MK-212 and fenfluramine with respect to their interactions with 5-HT antagonists. Considering 'high' (twice the ED_{50}) and 'low' (ED_{50}) doses of the two anorexigens, methergoline is the only antagonist effective against both compounds at both doses, cinanserin and cyproheptadine antagonizing only the high dose (present results and Clineschmidt *et al.*, 1974). The difference between methergoline and the other 5-HT antagonists cannot be attributed to inadequate doses of the latter, since, after raising the doses of cyproheptadine and cinanserin, pretreatment with these compounds was still without effect on the reduction in food consumption caused by the low dose of fenfluramine (Cline-

Table 2 Anorexic actions of MK-212 and amphetamine in rats treated with haloperidol

Pretreatment[a] *(mg/kg i.p.)*	*Treatment*[b] *(mg/kg i.p.)*	*Food intake*[c] *test day as % of control day*
Haloperidol (0.18)	Vehicle	109.7 ± 21.0
Vehicle	Amphetamine (3)	6.7 ± 8.8
Haloperidol (0.18)	Amphetamine (3)	41.5 ± 16.7*
Vehicle	MK-212 (3)	21.5 ± 11.5
Haloperidol (0.18)	MK-212 (3)	24.4 ± 14.0

[a] 2.5 h before treatment; [b] 30 min before presentation of food. [c] See Table 1. All values are mean ± s.d. of 10 animals per group.
* Significant difference compared to group pretreated with vehicle.

Table 3 Anorectic activity of MK-212 and amphetamine in rats injected intraventricularly with 5,6-dihydroxytryptamine (5,6-DHT)

			Food consumption (g)					
				5,6-DHT			*Ascorbate*	
Group	*Treatment*[a] *(mg/kg i.p.)*	*n*	*Control day*	*Test day*	*Test day as % of control day*	*Control day*	*Test day*	*Test day as % of control day*
1	MK-212 (3)	11	12.4 ± 2.2[b]	5.1 ± 2.0*	41.7 ± 15.5*	14.5 ± 2.9	2.9 ± 1.0	19.5 ± 5.1
2	MK-212 (1.5)	10	11.8 ± 2.9	8.9 ± 2.5	76.1 ± 18.7	14.1 ± 2.4	8.5 ± 2.2	62.3 ± 18.7
3	Amphetamine (2.5)	9	15.3 ± 3.5*	4.7 ± 2.0	31.7 ± 15.0	11.8 ± 2.0	3.2 ± 2.4	26.4 ± 20.3

			Brain monoamines (μg/g)[c]					
			5-HT		*NA*		*Dopamine*	
Group	*Treatment*[a] *(mg/kg i.p.)*	*n*	*5,6-DHT*	*Ascorbate*	*5,6-DHT*	*Ascorbate*	*5,6-DHT*	*Ascorbate*
1	MK-212 (3)	11	0.375* ± 0.044 −44%	0.667 ± 0.067	0.551 ± 0.059 −6%	0.589 ± 0.038	0.859* ± 0.062 −7%	0.923 ± 0.055
2	MK-212 (1.5)	10	0.468* ± 0.054 −36%	0.732 ± 0.088	0.589 ± 0.060 −2%	0.604 ± 0.040	0.899 ± 0.041 −2%	0.919 ± 0.031
3	Amphetamine (2.5)	9	0.525* ± 0.048 −32%	0.772 ± 0.034	0.580* ± 0.034 +18%	0.493 ± 0.071	0.908 ± 0.061 +5%	0.866 ± 0.052

[a] 30 min before presentation of food on the 14th day after pretreatment with 5,6-DHT or ascorbate. [b] All values in table are mean ± s.d. [c] Brains were removed 24 h after treatment. 5-HT = 5-hydroxytryptamine; NA = noradrenaline.
* Significantly different vs. ascorbate-treated control rats.

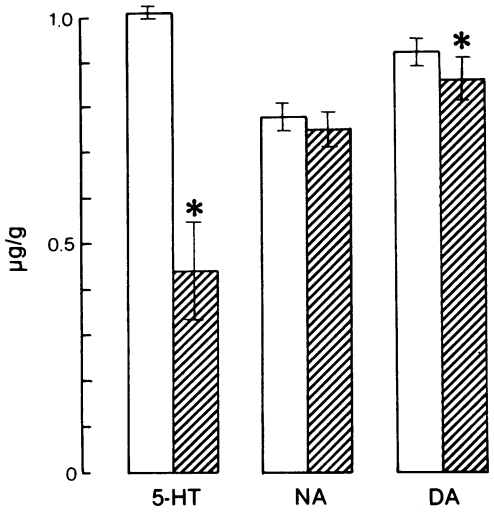

Figure 4 Whole brain concentrations of 5-hydroxytryptamine (5-HT), noradrenaline (NA) and dopamine (DA) after *p*-chloroamphetamine. The rats were treated 9 days before they were killed with *p*-chloroamphetamine (15 mg/kg i.p.). All data are expressed as the mean of 8 brains/group. Vertical lines show s.d. *Indicates a significant difference between treatment with *p*-chloroamphetamine (hatched) columns and the appropriate vehicle-treated (open columns) group

schmidt *et al.*, 1974). The inability of cinanserin and cyproheptadine to antagonize the low dose of MK-212 might indicate that a 5-HT-mimetic component is only involved at the high dose. However, such an interpretation requires that methergoline can also inhibit some undefined non-5-HT-mimetic mechanism operative at the low dose of MK-212. The selectivity of methergoline as an indoleamine antagonist (Ferrini & Glasser, 1965; Beretta *et al.*, 1965; Fuxe, Agnati & Everitt, 1975a; Sastry & Phillis, 1976) mitigates this possibility. In any event, the fact that three structurally dissimilar anti-5-HT agents significantly reduced the anorectic response to a high dose of MK-212 clearly points to an underlying 5-HT-like mode of action.

Receptors for 5-HT in peripheral tissues are unlikely to be important in the anorectic response to MK-212. Although MK-212 has a 5-HT-like effect on the rat uterus studied *in vitro*, it is 300-fold weaker than 5-HT itself (Lumma, Hartman, Saari, Clineschmidt & Torchiana, 1977). Despite its relatively weak peripheral 5-HT-mimetic effect, MK-212 has an ED_{50} for reducing food intake in the rat that is approximately three times lower than that of 5-HT (see Figure 3). Moreover, pretreatment with xylamidine to block peripheral receptors for 5-HT completely prevented the decrease in food consumption normally caused by 5-HT administered systemically, but xylamidine was totally ineffective as an antagonist of MK-212-elicited anorexia.

Table 4 Effect of pretreatment with *p*-chloroamphetamine (PCA) on the anorectic response to MK-212, fenfluramine and amphetamine

Pretreatment[a]	*Treatment*[b]	*Grams eaten*[c] *Control day*	*Test day*	*Test day as % of control day*
Vehicle	MK-212 (1.5)	13.0 ± 1.1[c]	4.9 ± 2.4	38.2 ± 20.1
PCA	MK-212 (1.5)	10.8 ± 1.9†	6.8 ± 2.0	64.2 ± 19.7*
Vehicle	MK-212 (3)	14.1 ± 3.7	4.0 ± 1.1	30.2 ± 11.6
PCA	MK-212 (3)	11.5 ± 2.5†	5.5 ± 1.9*	51.8 ± 19.6*
Vehicle	Fenfluramine (3)	12.2 ± 1.6	4.0 ± 1.7	34.3 ± 17.8
PCA	Fenfluramine (3)	11.8 ± 2.6	7.2 ± 1.9*	64.7 ± 24.1*
Vehicle	Fenfluramine (6)	14.2 ± 3.6	2.0 ± 2.3	16.7 ± 21.0
PCA	Fenfluramine (6)	11.8 ± 3.6	5.4 ± 2.1*	53.7 ± 28.4*
Vehicle	Amphetamine (1.5)	12.3 ± 1.4	5.2 ± 2.4	42.4 ± 19.2
PCA	Amphetumine (1.5)	13.1 ± 3.5	6.5 ± 1.8	51.5 ± 14.1
Vehicle	Amphetamine (3)	13.7 ± 3.6	1.0 ± 1.3	8.9 ± 12.5
PCA	Amphetamine (3)	10.0 ± 2.9†	2.9 ± 2.1*	35.0 ± 30.5*

[a] 15 mg/kg i.p. 9 days before treatment; [b] 30 min before presentation of food. [c] All values are mean ± s.d. of 10 rats per group.
† Significantly less than group pretreated with vehicle; * Significantly more than group pretreated with vehicle.

Table 5 Effect of an electrolytic lesion (19 to 21 days before treatment) in the area of the median raphé nucleus on the anorexigenic actions of MK-212, fenfluramine and amphetamine

			Food consumption (g)					
			Lesion			*Sham*		
Group	*Treatment[a] (mg/kg i.p.)*	*n*	*Control day*	*Test day*	*Test day as % of control day*	*Control day*	*Test day*	*Test day as % of control day*
1	Fenfluramine (6)	10	14.1 ± 3.4[b]	3.3 ± 2.9*	29.7 ± 34.8*	14.0 ± 2.9	0.0 ± 0.0	0.0 ± 0.0
2	MK-212 (3)	14[c]	13.6 ± 3.1	4.8 ± 2.6*	37.3 ± 21.5*	14.1 ± 3.2	1.1 ± 1.4	7.9 ± 9.7
3	Amphetamine (3)	8	15.3 ± 2.1*	6.0 ± 4.9*	37.6 ± 29.8*	12.9 ± 2.3	0.6 ± 0.9	5.0 ± 7.2

			Forebrain monoamines (μg/g)[d]					
			5-HT		*NA*		*Dopamine*	
Group	*Treatment[a] (mg/kg i.p.)*	*n*	*Lesion*	*Sham*	*Lesion*	*Sham*	*Lesion*	*Sham*
1	Fenfluramine (6)	10	0.359 ± 0.221* −48%	0.688 ± 0.196	0.562 ± 0.056 −1%	0.569 ± 0.085	1.385 ± 0.239 +2%	1.352 ± 0.178
2	MK-212 (3)	14[c]	0.370 ± 0.152* −45%	0.678 ± 0.108	0.529 ± 0.049 −7%	0.569 ± 0.072	1.382 ± 0.150 +1%	1.365 ± 0.173
3	Amphetamine (3)	8	0.421 ± 0.121* −37%	0.671 ± 0.069	0.435 ± 0.068* −17%	0.527 ± 0.057	1.432 ± 0.165 +6%	1.354 ± 0.086

[a] 30 min before feeding. [b] All data are expressed as mean ± s.d. [c] Noradrenaline and dopamine were determined in brains of 10 animals. [d] Brains were removed 48 h after treatment. 5-HT = 5-hydroxytryptamine, NA = noradrenaline.
* Lesioned animals differ significantly from sham operated controls.

To gain insight as to whether or not MK-212 might reduce food intake by releasing 5-HT from neurones in the brain, the compound was tested in rats whose level of 5-HT was decreased by destroying nerve terminals with 5,6-dihydroxytryptamine (Baumgarten, Bjorklund, Lachenmayer, Nobin & Stenevi, 1971), by a lesion of 5-hydroxytryptaminergic cell bodies in the median raphé nucleus or by administering *p*-chloroamphetamine, a 5-HT neurotoxin with an incompletely understood mode of action (Sanders-Bush, Bushing & Sulser, 1972; Fuller, Perry & Molloy, 1975; Neckers, Bertilsson, Koslow & Meek, 1976). Regardless of the method employed to lower 5-HT, the anorectic response to the high dose of MK-212 was reduced. This finding would appear to provide convincing evidence for an indirect 5-HT-mediated mode of action. However, consideration must be given to a point that tends to weaken the argument. Two procedures for lowering 5-HT, namely, lesion in the raphé nucleus and *p*-chloroamphetamine, resulted in a lessening of the anorectic activity of amphetamine. If one accepts the evidence for catecholamines in the mechanism whereby amphetamine causes anorexia (Weissman *et al.*, 1966; Abdalah, 1971; Holtzman & Jewett, 1971; Schulz & Frey, 1972; Clineschmidt *et al.*, 1974; Hollister *et al.*, 1975), a reduction in brain 5-HT would not be expected to diminish the effect of amphetamine. It is uncertain, therefore, whether or not the reduced anorectic activity of MK-212 after *p*-chloroamphetamine or lesion in the median raphé nucleus can be attributed to the decrease in brain 5-HT. The technique of placing lesions in the brain by electrolytic means is always questionable with respect to selectivity. As for *p*-chloroamphetamine, the rats consumed less food after receiving this compound, and it is possible that cross-tolerance to the two closely related drugs rather than the lower level of brain 5-HT might explain the reduction in amphetamine's anorectic action. Since MK-212 bears no obvious structural resemblance to *p*-chloroamphetamine, cross-tolerance between these two compounds seems less likely. It should be noted that amphetamine does affect 5-hydroxytryptaminergic neurones, e.g. increasing their rate of firing and accelerating the turnover of 5-HT (see review by Brase & Loh, 1975). Therefore, the results with *p*-chloroamphetamine and lesion in the raphé region could be evidence for implicating 5-HT in the mechanism of amphetamine's anorectic action. However, this proposition is difficult to reconcile with the failure of 5-HT antagonists, including methergoline, to reduce the anorectic response to amphetamine (Funderburk *et al.*, 1971; Jespersen & Scheel-Kruger, 1973; Clineschmidt *et al.*, 1974).

While it is generally agreed that fenfluramine produces anorexia in the rat by a 5-HT-like effect, the role of central 5-HT-containing neurones is controversial. Samanin *et al.* (1972) reported that an electrolytic lesion in the median raphé nucleus inhibited the anorectic response to fenfluramine without influencing the response to amphetamine. According to Sugrue, Goodlet & McIndewar (1975), fenfluramine-elicited anorexia was not reduced by placing a lesion in the median raphé nucleus. In the present study, the effects of both fenfluramine and amphetamine were less in lesioned animals. Fenfluramine caused a smaller decrease in food consumption in rats that had previously received a microinjection of 5,7-dihydroxytryptamine into the median raphé nucleus (Fuxe *et al.*, 1975b). 5,6-Dihydroxytryptamine given intraventricularly has been reported to antagonize (Clineschmidt, 1973) or have no effect (Sugrue *et al.*, 1975) on the magnitude of the anorectic response to fenfluramine. 5,7-Dihydroxytryptamine administered by the intracisternal route was ineffective in preventing anorexia provoked by fenfluramine (Hollister *et al.*, 1975). In agreement with the results presented here, Duhalt, Boulanger, Voisin, Malen & Schmitt (1975) reported a decrease of the anorectic activity of fenfluramine in rats pretreated with *p*-chloroamphetamine. If fenfluramine does act, at least in part, *via* the release of 5-HT from neurones in the central nervous system, it apparently requires the uptake mechanism present at the level of the neuronal membrane. Prior treatment with chlorimipramine, an inhibitor of the uptake pump (Carlsson, 1970), antagonizes the anorexigenic effect of fenfluramine (Jespersen & Scheel-Kruger, 1973; Clineschmidt *et al.*, 1974; Garattini *et al.*, 1975).

Despite the similarities between the mechanisms involved in the anorectic actions of fenfluramine and MK-212, there are some interesting differences between the two compounds. The anorectic response to MK-212 was not diminished by prior treatment with chlorimipramine. If MK-212 does act to some extent by releasing 5-HT, it apparently does not require the uptake mechanism in order to effect the release of the monoamine. Fenfluramine causes a long-lasting, *p*-chloroamphetamine-like, decrease in brain 5-HT (Harvey & McMaster, 1975; Clineschmidt, Totaro, McGuffin & Pflueger, 1976; Clineschmidt, Zacchei, Totaro, Pflueger, McGuffin & Wishousky, 1977). MK-212 does not share this property (unpublished findings).

References

ABDALAH, A.H. (1971). On the role of norepinephrine in the anorectic effect of d-amphetamine in mice. *Arch. int. Pharmacodyn. Ther.*, **192,** 72–80.

ANTON, A.H. & SAYRE, D.F. (1962). A study of the factors affecting the aluminum oxide trihydroxyindole procedure for the analysis of catecholamines. *J. Pharmac. exp. Ther.*, **138,** 360–375.

BAUMGARTEN, H.G., BJORKLUND, A., LACHENMAYER, L., NOBIN, A. & STENEVI, U. (1971). Long-lasting selective depletion of brain serotonin by 5,6-dihydroxytryptamine. *Acta physiol. scand.*, Suppl. **373,** 1–15.

BERETTA, C., GLASSER, A.H., NOBILI, M.B. & SILVESTRI, R. (1965). Antagonism of 5-hydroxytryptamime-induced bronchospasm in guinea pigs by 8β-carbobenzyloxy-aminomethyl-1-methyl-10-α-ergoline. *J. Pharm. Pharmac.*, **17,** 423–428.

BOGDANSKI, D.F., PLETSCHER, A., BRODIE, B.B. & UDENFRIEND, S. (1956). Identification and assay of serotonin in brain. *J. Pharmac. exp. Ther.*, **117,** 82–88.

BRASE, D.A. & LOH, H.H. (1975). Possible role of 5-hydroxytryptamine in minimal brain dysfunction. *Life Sci.*, **16,** 1005–1016.

CARLSSON, A. (1970). Structural specificity for inhibition of [^{14}C-]-5-hydroxytryptamine uptake by cerebral slices. *J. Pharm. Pharmac.*, **22,** 729–732.

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

CLINESCHMIDT, B.V. & LOTTI, V.J. (1974). Indoleamine antagonists: relative potencies as inhibitors of tryptamine- and 5-hydroxytryptophan-evoked responses. *Br. J. Pharmac.*, **50,** 311–313.

CLINESCHMIDT, B.V., HANSON, H.M., PFLUEGER, A.B. & McGUFFIN, J.C. (1977). Anorexigenic and ancillary actions of MK-212 (6-Chloro-2-[1-piperazinyl]-pyrazine; CPP). *Psychopharmacology* (in press).

CLINESCHMIDT, B.V., McGUFFIN, J.C. & WERNER, A.B. (1974). Role of monoamines in the anorexigenic actions of fenfluramine, amphetamine and p-chloromethamphetamine. *Eur. J. Pharmac.*, **27,** 313–323.

CLINESCHMIDT, B.V., TOTARO, J.A., McGUFFIN, J.C. & PFLUEGER, A.B. (1976). Fenfluramine: long-term reduction in brain serotonin (5-hydroxytryptamine). *Eur. J. Pharmac.*, **35,** 211–214.

CLINESCHMIDT, B.V., ZACCHEI, A.G., TOTARO, J.A., PFLUEGER, A.B., McGUFFIN, J.C. & WISHOUSKY, T.I. (1977). Fenfluramine and brain serotonin. *Ann. N.Y. Acad. Sci.* (in press).

COPP, F.C., GREEN, A.F., HODSON, H.F., RANDALL, A.W. & SIM, M.F. (1967). New peripheral antagonists of 5-hydroxytryptamine. *Nature, Lond.*, **214,** 200–201.

DUHAULT, J., BOULANGER, M., VOISIN, C., MALEN, C. & SCHMITT, H. (1975). Fenfluramine and 5-hydroxytryptamine. Part 2: Involvement of brain 5-hydroxytryptamine in the anorectic activity of fenfluramine. *Arzneim. Forsch.* (*Drug Research*), **25,** 1755–1762.

FERRINI, R. & GLASSER, A. (1965). Antagonism of central effect of tryptamine and 5-hydroxytryptophan by 1,6-dimethyl-8β-carbobenzyloxyamino-methyl-10-α-ergoline. *Psychopharmacologia*, **8,** 271–276.

FULLER, R.W., PERRY, K.W. & MOLLOY, B.B. (1975). Effect of 3-(p-trifluoromethylphenoxy)-N-methyl-3-phenylpropylamine on the depletion of brain serotonin by 4-chloroamphetamine. *J. Pharmac. exp. Ther.*, **193,** 796–803.

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,** 468–470.

FUXE, K., AGNATI, L. & EVERITT, B. (1975a). Effects of methergoline on central monoamine neurons. Evidence for a selective blockade of central 5-HT receptors. *Neurosci. Lett.*, **1,** 283–290.

FUXE, K., FARNEBO, L.O., HAMBERGER, B. & OGREN, S.O. (1975b). On the *in vivo* and *in vitro* actions of fenfluramine and its derivates on central monoamine neurons, especially 5-hydroxytryptamine neurons, and their relation to the anorectic activity of fenfluramine. Post-grad. *Med. J.*, **51** (Suppl. 1), 35–45.

GARATTINI, S., BUCZKO, W., JORI, A. & SAMANIN, R. (1975). The mechanism of action of fenfluramine. *Post-grad. Med. J.*, **51** (Suppl. 1), 27–35.

HARVEY, J.A. & McMASTER, S.E. (1975). Fenfluramine: evidence for a neurotoxic action on midbrain and a long-term depletion of serotonin. *Psychopharmac. Commun.*, **1,** 217–228.

HOLLISTER, A.S., ERVIN, G.N., COOPER, B.R. & BREESE, G.R. (1975). The roles of monoamine neural systems in the anorexia induced by (+)-amphetamine and related compounds. *Neuropharmacology*, **14,** 715–723.

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

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

JOY, R.J.T., EMMA, C.P. & MAYER, J. (1967). New rat feeding jar: use in study of relationships of food intake and body weight. *J. appl. Physiol.*, **23,** 589–590.

KÖNIG, J.F.R. & KLIPPEL, R.A. (1963). The Rat Brain, a Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem. Baltimore, Md: Williams and Wilkins.

LUMMA, W.C., HARTMAN, R.D., SAARI, W.S., CLINESCHMIDT, B.V. & TORCHIANA, M.L. (1977). Synthesis of 2-(1-piperazinyl)pyrazines with serotonin-like activity in the central nervous system. Abstracts of papers presented at the 174th National Meeting of the American Chemical Society, Chicago, Illinois (in press).

MAWSON, C. & WHITTINGTON, H. (1970). Evaluation of the peripheral and central antagonistic activities against 5-hydroxytryptamine of some new agents. *Br. J. Pharmac.*, **39,** 223P.

NECKERS, L.M., BERTILSSON, L., KOSLOW, S.H. & MEEK, J.L. (1976). Reduction of tryptophan hydroxylase activity and 5-hydroxytryptamine concentration in certain rat brain nuclei after p-chloroamphetamine. *J. Pharmac. exp. Ther.*, **196,** 333–338.

NOBLE, E.P., WURTMAN, R.J. & AXELROD, J. (1967). A simple and rapid method for injecting H^3-norepinephrine into the lateral ventricle of the rat brain. *Life Sci.*, **6,** 281–291.

PINDER, R.M., BROGDEN, R.N., SAWYER, P.R., SPEIGHT, T.M. & AVERY, G.S. (1975). Fenfluramine: A review of its pharmacological properties and therapeutic efficacy in obesity. *Drugs*, **10,** 241–323.

PORTER, C.C., TOTARO, J.A. & BURCIN, A. (1965). The relationship between radioactivity and norepinephrine concentrations in the brains and hearts of mice following administration of labeled methyldopa or 6-hydroxydopamine. *J. Pharmac. exp. Ther.*, **150,** 17–22.

RUBIN, B., PIALA, J.J., BURKE, J.C. & CRAVER, B.N. (1964). A new potent and specific serotonin inhibitor (SQ 10,643) 2'-(3-dimethylaminopropylthio)cinnamanilide hydrochloride: antiserotonin activity on uterus and on

gastrointestinal, vascular, and respiratory system of animals. *Arch. int. Pharmacodyn.*, **152,** 132–143.

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.

SANDERS-BUSH, E., BUSHING, J.A. & SULSER, F. (1972). Long-term effects of p-chloroamphetamine on tryptophan hydroxylase activity and on the levels of 5-hydroxytryptamine and 5-hydroxyindole acetic acid in brain. *Eur. J. Pharmac.*, **20,** 385–388.

SASTRY, B.S.R. & PHILLIS, J.W. (1976). Methergoline as a selective 5-hydroxytryptamine antagonist in the cerebral cortex. *Can. J. Physiol. Pharmac.*, **55,** 130–133.

SCHULZ, R. & FREY, H.H. (1972). Study into the mechanism of the anorectic action of amphetamine and its p-chloro analogue. *Acta Pharmac. Tox.*, **31,** Suppl. No. 1, 12.

STONE, C.A., WENGER, H.C., LUDDEN, C.T., STAVORSKI, J.M. & ROSS, C.A. (1961). Antiserotonin-antihistaminic properties of cyproheptadine. *J. Pharmac. exp. Ther.*, **131,** 73–84.

SUGRUE, M.F., GOODLET, I. & McINDEWAR, I. (1975). Failure of depletion of rat brain 5-hydroxytryptamine to alter fenfluramine-induced anorexia. *J. Pharm. Pharmac.*, **27,** 950–953.

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

(Received September 27, 1977.)