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Evidence for a difference in mechanism of action between fenfluramine- and amphetamine-induced anorexia

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The influence of drugs, active on 5-hydroxytryptamine (5-HT) mechanisms, has been examined on the anorexigenic activity of fenfluramine and (+)-amphetamine in rats trained to consume their daily food ration during 6 h. Chlorimipramine, which inhibits the re-uptake mechanisms in central 5-HT neurons, and the 5-HT blocking drugs methergoline and methysergide were used. Fenfluramine, 7·5 mg kg^{-1}, and amphetamine, 2·5 mg kg^{-1}, given $\frac{1}{2}$ h before feeding reduced the food intake during the following 2 h to approximately 40% compared with control days. Pretreatment with methergoline in the optimal dose (1 mg kg^{-1}) produced only a weak but significant antagonism to amphetamine anorexia, whereas the fenfluramine anorexia was strongly antagonized by methergoline in all doses tested (0·3, 1 and 3 mg kg^{-1}). Methysergide (0·1, 0·3, 1 and 3 mg kg^{-1}) showed no significant antagonism against amphetamine or fenfluramine anorexia. Chlorimipramine produced a strong antagonistic effect to the fenfluramine anorexia, but showed no antagonism against amphetamine. In contrast the highest dose of chlorimipramine (20 mg kg^{-1}) potentiated amphetamine anorexia. The present results together with other evidence discussed support the conclusion that 5-HT mechanisms are involved in fenfluramine anorexia, whereas amphetamine anorexia seems mainly correlated with catecholamine dependent mechanisms.

Fenfluramine, a trifluoromethyl substituted analogue of amphetamine, produces anorexia like amphetamine but without amphetamine-like stimulant effects (Le Douarec, Schmitt & Laubie, 1966; Colmore & Moore, 1966;Yelnosky & Lawler, 1970; Tang & Kirch, 1971). The central stimulant effects of amphetamine are mainly correlated with an interaction on the adrenergic system (Randrup & Munkvad, 1966; Carr & Moore, 1970; Fuxe & Ungerstedt, 1970; Scheel-Krüger, 1971, 1972). The role of brain catecholamines in control of feeding behaviour seems well established (see review by Booth, 1968) but recent evidence also indicates a role of brain 5-hydroxytryptamine (5-HT) (Perez-Cruet, Tagliamonte, & others, 1972; Samanin, Ghezzi & others, 1972; Jespersen & Scheel-Krüger, 1970; Funderburk, Hazelwood & others, 1971; Opitz, 1967).

It has been shown that α-methyltyrosine, a specific inhibitor of the biosynthesis of the catecholamines, in doses which alone have no effect on food intake, antagonizes the anorectic activity of amphetamine (Weissman, Koe & Tenen, 1966; Abdallah, 1971; Holtzman & Jewett, 1971).

There is evidence that fenfluramine interacts with 5-HT mechanisms (Opitz, 1967; Jespersen & Scheel-Krüger, 1970; Funderburk & others, 1971; Southgate, Mayer

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& others, 1971) and decreases brain 5-HT and increases its turnover rate (Duhault & Verdavainne, 1967; Costa, Groppetti & Revuelta, 1971; Tagliamonte, Tagliamonte & others, 1971).

We have examined the influence of drugs active on 5-HT mechanisms on the anorexigenic activity of fenfluramine and amphetamine.

The 5-HT receptor blocking drugs methergoline and methysergide (Ferrini & Glässer, 1965; Corne & Pickering, 1967; Mawson & Whittington, 1970) and chlorimipramine which inhibits the re-uptake mechanism in central 5-HT neurons (Carlsson, Corrodi & others, 1969; Meek, Fuxe & Andén, 1970; Ross, Renyi & Ögren, 1972) were used.

MATERIALS AND METHODS

Female Wistar rats, 120–130 g, kept in separate cages (40 × 20 × 16 cm) in a room at 21–23°, artificially illuminated from 6 a.m. to 6 p.m. and then in the dark, were trained to eat all their food during a daily 6 h period. Water was freely available. After two weeks of training, when the body weight and food intake were stabilized on a slightly increasing level, cross-over experiments were made once a week. All drugs were dissolved in 0·9% saline and administered intraperitoneally in a volume of 1 ml per 100 g body weight.

The rats were pretreated with chlorimipramine hydrochloride, methysergide bimaleate or saline 1 h before normal feeding or with methergoline or its solvent (0·05% ascorbic acid in saline) 3½ h before feeding. (±)-Fenfluramine hydrochloride, (+)-amphetamine sulphate or saline was given 30 min before feeding.

The food consumption over 2 h was determined for each rat and expressed in percentage of the average food intake of the same rat during the corresponding 2 h of the two preceding days (control days).

RESULTS AND DISCUSSION

The effects on food intake of the various treatments are presented in Figs 1–3. Fig. 1 shows that methergoline (0·3, 1 and 3 mg kg^{-1}) shows a strong and significant antagonism to the anorexia produced by fenfluramine, 5 and 7·5 mg kg^{-1}. In the experiment with fenfluramine, 5 mg kg^{-1}, the food intake returned to normal. The effect of fenfluramine, 7·5 mg kg^{-1} was antagonized by methergoline, 1 mg kg^{-1}, from 35 to 80% of the control value. The food intake for control rats was approximately 8 g in the 2 h test period. This effect agrees exactly with the findings of Funderburk & others (1971), concerning the effect of 1 mg kg^{-1} methergoline against the anorexia caused by 8 mg kg^{-1} fenfluramine.

Methergoline in this optimal dose (1 mg kg^{-1}) produced only a weak but significant antagonism to the amphetamine anorexia (from 42 to 59% of the control value, $P < 0{\cdot}05$), whereas the other doses tested (0·3 and 3 mg kg^{-1}) produced no significant change. This might indicate some effect on the 5-HT system by amphetamine in agreement with the biochemical findings of Reid (1970) and Schubert & Sedvall (1972).

Methysergide (0·1, 0·3, 1 and 3 mg kg^{-1}) did not antagonize fenfluramine or amphetamine significantly but the 0·1 mg kg^{-1} dose enhanced fenfluramine anorexia (Fig. 2).

This result might be explained in view of the great difference between the two drugs in central anti-5-HT potency as measured in mice and rats. Ferrini & Glässer

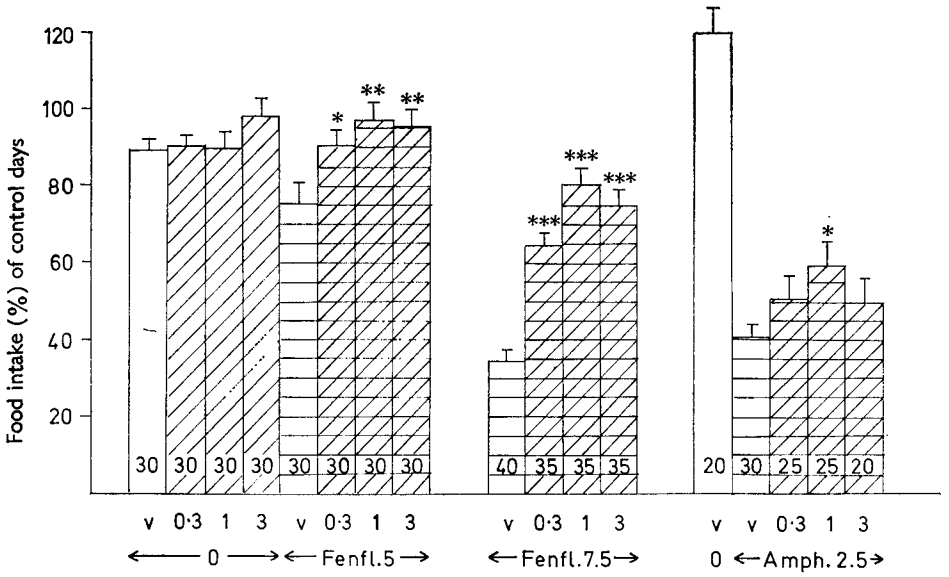

FIG. 1. The effect on food intake of fenfluramine (fenfl.), (+)-amphetamine (amph.), saline (O) and methergoline or combinations of methergoline with fenfluramine or (+)-amphetamine. Methergoline or the vehicle (v = 0·05% ascorbic acid in saline), was given i.p. 3½ h before feeding and fenfluramine, (+)-amphetamine or saline (i.p.) ½ h before feeding. Figures within the columns show the number of rats. Vertical bars indicate the standard error of mean and asterisks mean significant difference from rats treated with anorexic only. Abscissa: upper scale, methergoline mg kg^{-1}; lower scale, fenfluramine or amphetamine mg kg^{-1}. Open columns, vehicle + saline; horizontal bars, vehicle + fenfluramine or amphetamine; diagonal bars, methergoline + saline; horizontal and diagonal bars, methergoline + fenfluramine or amphetamine. (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ (Student's t-test)).

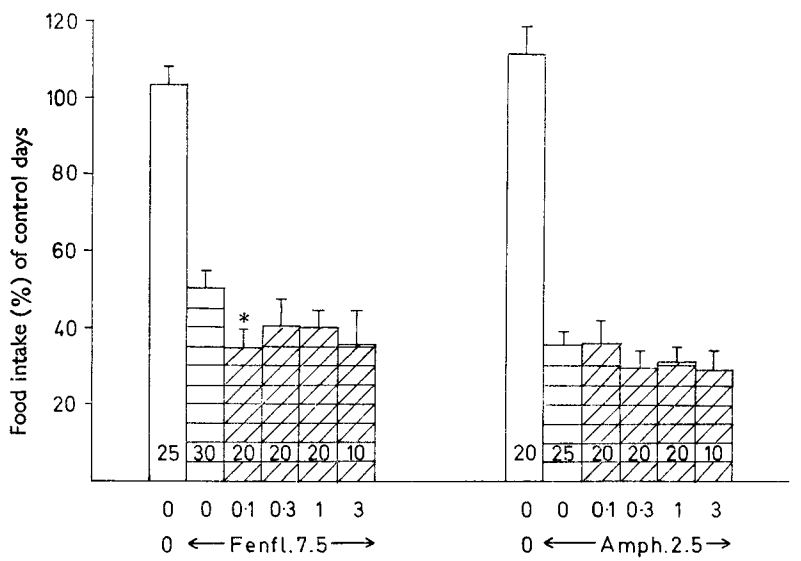

FIG. 2. The effect on food intake of fenfluramine (fenfl.), (+)-amphetamine (amph.) and saline (O) or combinations of methysergide with fenfluramine or (+)-amphetamine. Methysergide or saline was given i.p. 1 h before feeding and fenfluramine, (+)-amphetamine or saline i.p. ½ h before feeding. Figures within the columns show the number of rats. Vertical bars indicate the standard error of mean and the asterisk means significant difference ($P < 0.05$ (Student's t-test)) from rats treated with fenfluramine only. Abscissa: upper scale, methysergide mg kg^{-1}; lower scale, fenfluramine or amphetamine mg kg^{-1}. Open columns, saline + saline; horizontal bars, saline + fenfluramine or amphetamine; horizontal and diagonal bars, methysergide plus fenfluramine or amphetamine.

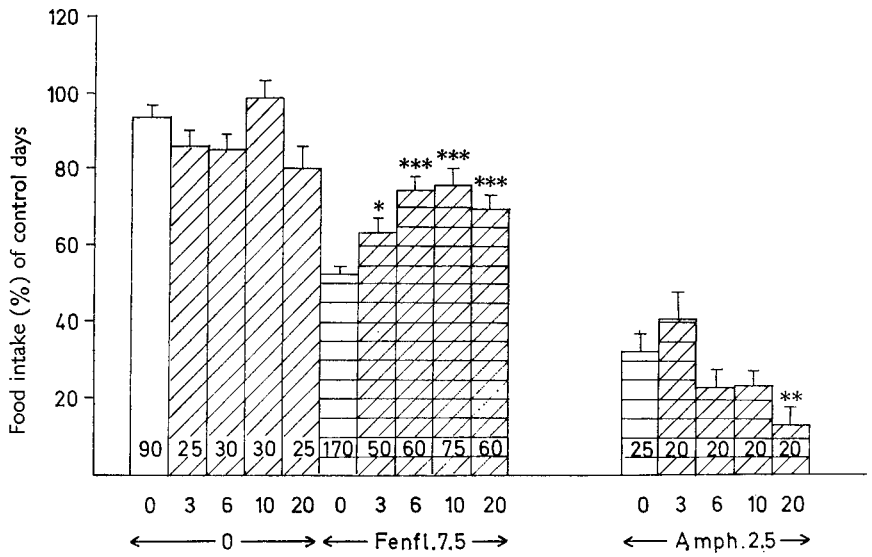

FIG. 3. The effect on food intake of fenfluramine (fenfl.), (+)-amphetamine (amph.), saline (O) and chlorimipramine or combinations of chlorimipramine with fenfluramine or (+)-amphetamine. Chlorimipramine or saline was given i.p. 1 h before feeding and fenfluramine, (+)-amphetamine or saline i.p. ½ h before feeding. Figures within the columns show the number of rats. Vertical bars indicate the standard error of mean and asterisks mean significant difference from rats treated with anorexic only. Abscissa: upper scale, chlorimipramine mg kg^{-1}; lower scale, fenfluramine or amphetamine mg kg^{-1}. Open columns, saline + saline; horizontal bars, saline + fenfluramine or amphetamine; diagonal bars, chlorimipramine + saline; horizontal and diagonal bars, chlorimipramine + fenfluramine or amphetamine. (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. Student's t-test).

(1965) and Mawson & Whittington (1970) found methergoline to be 10–30 times more active than methysergide.

Southgate & others (1971) used very high doses of methysergide (20–40 mg kg^{-1}) to antagonize significantly some 5-HT-like actions of fenfluramine in mice pretreated with a monoamine oxidase inhibitor.

The difference between methergoline and methysergide might also be due to species differences since we previously found that methysergide antagonized fenfluramine hypothermia, anorexia and behavioural effects in dogs (Jespersen & Scheel-Krüger, 1970).

At present, it is debatable whether fenfluramine produces its anorectic effect indirectly by releasing brain 5-HT or via a direct receptor stimulant effect (Opitz, 1967; Funderburk & others, 1971).

A close correlation has been established between a 5-HT receptor blocking effect and the antagonism to fenfluramine-induced anorexia (Jespersen & Scheel-Krüger, 1970 and present paper; Funderburk & others, 1971). However, *p*-chlorophenylalanine, an inhibitor of the biosynthesis of brain 5-HT, has failed to antagonize the fenfluramine anorexia (Opitz, 1967; Funderburk & others, 1971).

The biochemical studies might point to an indirect action of fenfluramine since it reduces brain 5-HT and increases its turnover rate (Duhault & Verdavainne, 1967; Costa & others, 1971; Tagliamonte & others, 1971).

Samanin & others (1972), have shown that a lesion of the midbrain raphe, which causes a selective degeneration of 5-HT neurons in the brain, does not affect food

intake in rats. However, the anorectic effect of fenfluramine was abolished in midbrain raphe lesioned animals whereas amphetamine was still able to produce the usual decrease of food intake. This result suggests that the anorectic effect of fenfluramine but not that of amphetamine could be due to a release of brain 5-HT.

Chlorimipramine (3, 6, 10 and 20 mg kg^{-1}) produced a dose-related antagonism to the fenfluramine anorexigenic effect, but no significant antagonism to amphetamine (Fig. 3), on the contrary, the highest dose of chlorimipramine (20 mg kg^{-1}) significantly increased amphetamine anorexia. Our findings that chlorimipramine inhibits the fenfluramine anorexia points to an indirect action of fenfluramine.

Concerning the mechanism of action of this antagonistic effect, Carlsson & others (1969), Meek, Fuxe & Carlsson (1971) and Schrold (1972), have shown that tricyclic antidepressant drugs which inhibit the re-uptake mechanism in 5-HT neurons protect against the biochemical and behavioural effects of some amphetamine derivatives, H75/12 (4-methyl-α-ethyl-*m*-tyramine) and *p*-chloromethamphetamine, which produce depletion of brain 5-HT. Squires (Ferrosan Research Laboratories A/S, Copenhagen Denmark, in preparation) has shown that chlorimipramine (10 mg kg^{-1}) produces complete protection against the fenfluramine-induced decrease in brain 5-HT.

It has been suggested that chlorimipramine prevents the accumulation of these amphetamine derivatives in 5-HT neurons or inhibits the release of 5-HT by these drugs (Carlsson & others, 1969; Meek & others, 1971; Schrold, 1972).

It seems reasonable to conclude that 5-HT mechanisms are involved in the fenfluramine anorexia while on the other hand amphetamine anorexia seems mainly correlated with catecholamine dependent mechanisms.

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