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Inhibition of drug-induced anorexia in rats by methysergide

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Iproniazid was found to reduce food consumption in fasting rats. Combined treatment of iproniazid with tryptophan resulted in a significantly greater anorexic action whilst tryptophan alone had no effect on food consumption. Iproniazid treatment was associated with a significant increase in brain 5-hydroxytryptamine (5-HT) concentration but in association with tryptophan higher brain 5-HT concentrations were recorded. The anorexic action of the iproniazid-tryptophan combination was antagonized in a dose-dependent fashion by methysergide. Equivalent levels of anorexia induced by fenfluramine and mazindol were similarly antagonized by methysergide in a dose-related manner. The results suggest a common role of 5-HT in the inhibition of eating behaviour in fasting rats when anorexia is induced by iproniazid, fenfluramine or mazindol, sensitive to a specific 5-HT antagonist.

Daily administration of the monoamine oxidase inhibitor mebanazine (15 mg kg^{-1} oral) to adult rats over 6 weeks resulted in a significant reduction in mean daily food intake and virtual elimination of weight gain (Barrett, 1969). Anorexic activity was also demonstrated acutely although no hypothesis was advanced to explain the observations. There was, however, a clearly different pattern of activity compared to that of amphetamine. Recent reports in this Journal have emphasized the dissociation of the time course of action of amphetamine and fenfluramine reminiscent of that of amphetamine and mebanazine (Blundell, Campbell & others, 1975). In addition there are clear implications of the involvement of 5-hydroxytryptamine (5-HT) in the anorexic action of fenfluramine, not shared by amphetamine (Samanin, Ghezzi & others, 1972; Blundell & Leshem, 1975).

Pretreatment of rats with the monoamine oxidase inhibitor iproniazid resulted in a greater rise in brain 5-HT concentration than in brain noradrenaline (Passonen & Kärki, 1959). It seemed possible that the anorexic action of mebanazine might derive from altered 5-HT availability more closely resembling the biochemical consequences of fenfluramine administration than those of amphetamine. Inhibition of the appetite suppressant activity of fenfluramine has been reported to follow pre-treatment with 5-HT antagonists (Jespersen & Scheel-Krüger, 1973; Blundell, Latham & Leshem, 1973). The present investigation attempted to determine the effects of iproniazid on eating behaviour in rats and relate any change to brain 5-HT concentrations. In addition any potential interaction with the specific 5-HT antagonist, methysergide, was studied and compared with fenfluramine and the new imidazo-isoindole derivative, mazindol, an anorexic agent without monoamine oxidase inhibitory activity (Gogerty, Houlihan & others, 1968).

METHODS

Male albino rats of a Wistar strain, 250–350 g, were maintained in stock cages on a standard laboratory diet (pellets) with free access to water. For food intake experiments the animals were housed singly, without food but with water, for 18 h before presentation with 100 g of standard diet in a food container. Food consumption was estimated by difference after 4 h taking care to collect any spillage. Groups of at least 5 rats were used for any individual treatment and animals were not used twice within any 7 day period. Drugs were administered by intraperitoneal injection in volumes of 0·1 ml/100 g 1 h before allowing access to food. All procedures were standardized to the same time each day to minimize any diurnal variations. Within any individual experiment the weight range did not exceed 20 g.

Brain 5-hydroxytryptamine (5-HT) was measured 2 h after saline or drug injections, injection time being the same as that for animals in the food intake experiments. The 2 h interval was chosen to correspond with the time of maximal change observed by Spector, Shore & Brodie (1960). For estimation of brain 5-HT, rats were stunned before decapitation and the brains rapidly removed. The tissue analysed was that rostral to the pons excluding the cerebellum corresponding to the "tel-diencephalon" of Costa, Groppetti & Revuelta, (1971) or the "fore-brain" of Samanin & others (1972). After blotting and weighing, brain tissue was homogenized in ice-cold 0·4 M perchloric acid and 5-HT was extracted and measured by the method of Snyder, Axelrod & Zweig (1965) subject to two modifications (a) substitution of cyclohexane for heptane (b) raising ninhydrin concentration from 0·1 to 0·2 M which improved the stability of the fluorophore. Recovery of added 5-HT was 95–105%.

The drugs used were 5-hydroxytryptamine creatinine sulphate, L-tryptophan (Sigma), iproniazid phosphate (Roche), methysergide and mazindol (Sandoz) and fenfluramine HCl (Servier). Solutions were prepared freshly in 0·9% NaCl solution (saline) except for mazindol which was dissolved in 10% citric acid.

Mean food intake was calculated for each group of rats, together with the standard error of the mean. Results from more than one group for any individual treatment have been pooled and recalculated where there was no statistically significant difference between the mean values for each group. Statistical significance between mean values was derived from Student's *t*-test.

RESULTS

The amount of food consumed in a 4 h period following 18 h of fasting, in groups of rats receiving a saline injection, ranged from a mean of 6·5 to 9·7 g, the value being proportional to body weight. Pretreatment with iproniazid alone appeared to induce a dose-dependent inhibition of food intake although at the lowest dose tested (50 mg kg^{-1}) a modest increase was observed (Table 1). Administration of tryptophan alone had no effect on the food intake of fasted rats but in combination with iproniazid there was a highly significant potentiation of the anorexic effect of the monoamine oxidase inhibitor (Table 1).

The brain 5-HT concentration was some 22% higher in rats which had been fasted for 18 h when compared with values for animals which had been allowed food up till sampling time. The rats treated with iproniazid alone showed a 19% increase in brain 5-HT compared to fasted controls but the addition of tryptophan to the pretreatment resulted in a 50% elevation in the concentration of the amine (Table 2).

Table 1. *Food intake in a 4 h period following a 18 h fast in rats pretreated (i.p.) with saline, iproniazid, tryptophan or a combination of tryptophan and iproniazid at various dose levels 1 h before access to food.*

No. of rats	Treatment	Dose (ml or mg kg^{-1})	4 h food intake (g) (mean ± s.e.m.)	% change	$P<$ *vs* saline
12	Saline	1·0	9·7 ± 0·5	—	—
12	Iproniazid	50	11·6 ± 0·5	+19	0·02
12	Iproniazid	100	8·1 ± 0·6	−17	0·05
12	Iproniazid	200	4·4 ± 0·3	−54	0·001
12	Saline	1·0	6·8 ± 0·6	—	—
6	Iproniazid	100	5·9 ± 1·2	−13	NS
6	Tryptophan	25	6·7 ± 0·8	−2	NS
6	Iproniazid + tryptophan	100 + 25	2·3 ± 0·3	−67	0·001

NS = not significant.

Over the dose-range of 1·25–5·0 mg kg^{-1}, methysergide did not exert any significant effect on the amount of food consumed by fasting rats (Table 3). As in the earlier experiment, the combination of tryptophan and iproniazid resulted in a marked reduction of 4 h food intake. Inclusion of methysergide in the pretreatment regime resulted in the animals eating more food than when receiving iproniazid and tryptophan without the 5-HT antagonist. This inhibition of anorexic activity was dose-dependent and at each dose level of methysergide the increase in food intake was statistically significant when compared to the iproniazid plus tryptophan group (Table 3).

From preliminary experiments, dose levels of fenfluramine and mazindol were selected (16 and 8 mg kg^{-1} respectively) which might be expected to induce a similar sub-maximal anorexic effect, over the 4 h food access period, to that found with iproniazid and tryptophan. Rats pretreated with graded doses of methysergide and a standard dose of fenfluramine demonstrated a similar dose-dependent reversal of anorexic activity (Table 4). Essentially similar results were obtained when studying the interaction between methysergide and mazindol i.e. a dose-dependent reduction in the inhibition of food consumption produced by mazindol alone. It may be of interest to record that the combination of mazindol 8 mg kg^{-1} with methysergide 5 mg kg^{-1} induced a characteristic hyperactivity lasting about 2 h in all rats so treated,

Table 2. *Concentration of 5-HT in brain of rats either allowed free access to food or fasted for 18 h before treatment (i.p.) with saline, iproniazid or a combination of tryptophan and iproniazid: animals were killed 2 h after injections.*

No. of rats	Nutritional status	Treatment	Dose (ml or mg kg^{-1})	Brain (wet wt g) mean ± s.e.m.	5-HT (μg g^{-1} wet wt) mean ± s.e.m.	$P<$ control
6	Fed	Saline	1·0	1·38 ± 0·02	0·37 ± 0·02	—
6	Fasted	Saline	1·0	1·42 ± 0·03	0·45 ± 0·01	0·02
9	Fasted	Saline	1·0	1·40 ± 0·02	0·42 ± 0·01	—
9	Fasted	Iproniazid	100	1·43 ± 0·03	0·50 ± 0·01	0·001
9	Fasted	Iproniazid + tryptophan	100 + 25	1·34 ± 0·02	0·63 ± 0·04	0·001

Table 3. *Food intake in a 4 h period following a 18 h fast in rats pretreated (i.p.) with saline, methysergide or a combination of iproniazid plus tryptophan with or without graded doses of methysergide 1 h before access to food.*

No. of rats	Treatment	Dose (ml or mg kg^{-1})	4 h food intake (g)	% change	$P<$ vs saline	$P<$ vs iproniazid + tryptamine
12	Saline	1·0	6·5 ± 0·5	—	—	—
12	Methysergide	1·25	6·9 ± 0·7	+6	NS	—
12	Methysergide	2·5	6·0 ± 0·9	−8	NS	—
12	Methysergide	5·0	6·3 ± 0·6	−3	NS	—
24	Saline	1·0	8·1 ± 0·4	—	—	—
23	Iproniazid + tryptophan	100 25	1·8 ± 0·3	−78	0·001	—
12	Iproniazid + tryptophan + methysergide	100 25 1·25	4·0 ± 0·7	−52	0·001	0·01
18	Iproniazid + tryptophan + methysergide	100 25 2·5	5·1 ± 0·3	−35	0·001	0·001
18	Iproniazid + tryptophan + methysergide	100 25 5·0	6·0 ± 0·4	−26	0·01	0·001

which was not observed in any other treatment group. This response did not impair eating behaviour since the value for this group was not significantly different from the saline controls.

To facilitate a comparison of the inhibitory action of methysergide on the anorexic action of the three treatments, the percentage reduction in food intake suppression has been calculated from the mean values of each relevant group. The relation between percentage inhibition of anorexia and the logarithm of the dose of methysergide was linear for all three treatments, the slopes for mazindol and fenfluramine being

Table 4. *Food intake in a 4 h period following a 18 h fast in rats pretreated (i.p.) with saline, fenfluramine and mazindol or a combination of fenfluramine or mazindol with graded doses of methysergide, 1 h before access to food.*

No. of rats	Treatment	Dose (ml or mg kg^{-1})	4 h food intake (g)	% change	$P<$ vs saline	$P<$ vs drug alone
10	Saline	1·0	8·4 ± 0·3	—	—	—
8	Fenfluramine	16	2·3 ± 0·6	−73	0·001	—
10	Fenfluramine + methysergide	16 1·25	2·9 ± 0·6	−66	0·001	NS
10	Fenfluramine + methysergide	16 2·5	4·6 ± 0·9	−46	0·001	0·005
10	Fenfluramine + methysergide	16 5·0	6·0 ± 1·0	−29	0·05	0·01
12	Saline	1·0	8·1 ± 0·4	—	—	—
11	Mazindol	8	3·0 ± 0·4	−64	0·001	—
12	Mazindol + methysergide	8 1·25	4·3 ± 0·8	−47	0·001	NS
12	Mazindol + methysergide	8 2·5	5·6 ± 1·0	−31	0·05	0·02
11	Mazindol + methysergide	8 5·0	6·8 ± 1·0	−16	NS	0·01

approximately parallel whilst that for iproniazid and tryptophan rather less steep (Fig. 1). No statistical analysis was performed bearing in mind the inherent variation in the raw data and the availability of only three points for each curve. The basal anorexia for iproniazid plus tryptophan was 78% compared with 73% for fenfluramine and 64% for mazindol. It appears that methysergide is a more effective antagonist to mazindol than to fenfluramine but full dose response curves in the presence and absence of a fixed dose of antagonist would be required to establish this implication with certainty.

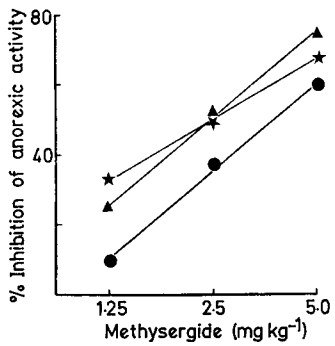

FIG. 1. The percentage inhibition of the anorexic action of iproniazid (100 mg kg^{-1}) L-tryptophan (25 mg kg^{-1}) (★——★); fenfluramine (16 mg kg^{-1}) (●——●); and mazindol (8 mg kg^{-1}) (▲——▲) by varying doses of methysergide in fasting rats during 4 h feeding tests.

DISCUSSION

A dose-dependent inhibition of appetite was evident following iproniazid pretreatment at dose levels in excess of 50 mg kg^{-1}. It has been shown that iproniazid is rapidly taken up by rat brain following intraperitoneal administration and that a 90% inhibition of brain monoamine oxidase activity was demonstrable within 2 h (Hess, Weisbach & others, 1958). This time interval coincides with the maximal rise in brain 5-HT concentration to levels sustained over the next 6 h (Spector & others, 1960). At 100 mg kg^{-1} iproniazid treatment was associated with a 19% increase in brain 5-HT ($P<0{\cdot}001$; d.f. 16) and a 17% decrease in food intake ($P<0{\cdot}05$; d.f. 22). Whilst a greater inhibition of appetite was evident at 200 mg kg^{-1}, an alternative way of boosting brain 5-HT concentration was sought since other central nervous system effects may interfere with food intake measurements at this dose concentration. Administration of a biosynthetic precursor of 5-HT, L-tryptophan, has been shown to raise the brain amine concentration (Fernstrom & Wurtman, 1971). We chose to combine tryptophan and iproniazid treatment and found a statistically significant gain in anorexic activity when compared with the same dose of iproniazid alone (67 *vs* 13%; $P<0{\cdot}01$; d.f. 10). The combined treatment also resulted in a larger increase in brain 5-HT content of rats than did the single treatment (50 *vs* 19%; $P<0{\cdot}001$; d.f. 16). Such results would be consistent with two provisional conclusions (a) that the anorexic action of an inhibitor of monoamine oxidase is related to an altered rate of 5-HT metabolism and (b) that the degree of anorexia is proportional to brain 5-HT concentration.

Direct administration of 5-HT into the cerebral ventricles of rats has been shown to reduce food intake in fasted animals (Kruk, 1973). Whilst the dose applied was large (100 μg 5-HT cf. total brain content of less than 1 μg), the fact that the effect was

largely abolished by pretreatment (i.p.) with cyproheptadine a specific antagonist of 5-HT (Stone, Wenger & others, 1961), implied a selective serotoninergic mechanism. The present results extend this observation since not only was brain 5-HT concentration proportional to inhibition of food intake but the anorexic action of the iproniazid + tryptophan combination was antagonized effectively by methysergide. Further, the antagonism produced by methysergide was clearly dose dependent and yielded a linear logarithmic relationship. These results decrease the probability of decreased noradrenaline metabolism playing an important role in anorexia following monoamine oxidase inhibition.

Interactions between 5-HT antagonists and anorexic agents have been reported earlier. In dogs, intravenous methysergide reversed fenfluramine-induced anorexia (Jesperson & Scheel-Krüger, 1970). In rats, intraperitoneal administration of methergoline also reduced the inhibition of food intake due to fenfluramine (Funderburk, Hazelwood & others, 1971), subsequently confirmed by Jespersen & Scheel-Krüger (1973). The latter authors however, failed to observe a significant effect of methysergide, in doses up to 3 mg kg^{-1}, against fenfluramine or amphetamine-induced anorexia. Kruk (1973) found that central administration of desethylfenfluramine produced a cyproheptadine-sensitive inhibition of food consumption in fasting rats. At a dose level of 5 mg kg^{-1} (s.c.), Blundell & others (1973) showed that methysergide could apparently antagonize or facilitate fenfluramine's anorexic activity in both fasted and satiated rats, depending on the duration of the feeding test or the time interval between drug injection and access to food. During the first hour of feeding there was a marked inhibition but over 8 h a potentiation and an indication that the change-over was occurring at 4 h. We have been unable to confirm such a "biphasic" effect noting only an inhibition of fenfluramine-induced anorexia by methysergide. Relative pharmacokinetic data should be known before attempting explanations. Under our conditions, methysergide reversal of the anorexic action of fenfluramine was dose-dependent and more impressive than that claimed for methergoline by Jespersen & Scheel-Krüger (1973). In addition, a similar dose-dependent inhibition of the anorexic action of mazindol was observed in the presence of methysergide suggesting that a common component, involving 5-hydroxytryptamine or its receptors, may contribute to the anorexia produced by both mazindol and fenfluramine.

A substantial body of evidence supports the view that the mode of action of fenfluramine is different from that of the amphetamines (see Blundell & Leshem, 1975 for references). It is less clear, however, to what extent the anorexic action is direct, e.g. by stimulation of 5-HT receptors or indirect, e.g. release of 5-HT from neurons or an inhibition of re-uptake. The effects may be independent or additive in relation to the production of anorexia. β-Methoxylated analogues of fenfluramine retain anorexic activity but lose their ability to decrease brain 5-HT content (Cattabeni, Revuelta & Costa, 1972). Using blood platelets as a model for tryptaminergic nerve endings, Buczko, De Gaetano & Garattini (1975) have shown a dual effect of fenfluramine on the uptake and release of 5-HT, possibly explaining depletion of central stores (Duhault & Verdavainne, 1967) but there is little direct evidence that depletion *per se* is responsible for anorexia. Selective elimination of 5-HT-containing neurons by lesion techniques (Samanin & others, 1972) or chemical means (Clineschmidt, 1973) abolishes the anorexic action of fenfluramine but the effect of 5-HT itself under such conditions is not known. Depletion of brain 5-HT by *p*-chlorophenylalanine does not prevent the inhibition of eating by fenfluramine (Opitz, 1967; Funderburk

& others, 1971) which may argue a direct tryptaminergic action. The present studies do not permit a distinction between a mechanism involving release of 5-HT from specific neurons or a direct activation of 5-HT receptors which might account for the anorexic action of fenfluramine and mazindol. Whatever distinctions there may be in the pharmacological profiles of fenfluramine and mazindol (Hadler, 1972) they appear to be equally subject to antagonism by methysergide. The degree of parallelism between the nature of the dose-dependent inhibition of anorexia by methysergide, whether due to iproniazid, fenfluramine or mazindol is consistent with the hypothesis that direct occupation of 5-HT receptors in the brain plays a common role in the production of the pharmacological response. Clinical reports of weight gain in patients receiving cyproheptadine (Noble, 1969) and pizotifen (BC 105; Hughes & Foster, 1971), both antagonists of 5-hydroxytryptamine, add further interest to this hypothesis.

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