nalorphine. The possibility therefore exists that this variable response to LAAM in monkeys could be due to the differential rates of formation of free and conjugated *p*-hydroxyacetylbisnormethadol or some other metabolite.

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Effects of Lilly 110140, a specific inhibitor of 5-hydroxytryptamine uptake, on food intake and on 5-hydroxytryptophan-induced anorexia. Evidence for serotoninergic inhibition of feeding

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A number of recent studies suggest that an inhibitory serotoninergic system is involved in the control of food intake. In rats intraventricular injection of 5-hydroxytryptamine (5-HT) has been found to inhibit food intake, an effect which is blocked by the 5-HT antagonist cyproheptadine (Kruk, 1973). Furthermore, cyproheptadine has been reported to increase body weight, food intake and subjective feelings of hunger in man (Silverstone & Schuyler, 1975) and body weight in rats (Gosh & Parvarty, 1973). In addition, a large body of evidence indicates that the anorectic properties of fenfluramine are mediated by direct or indirect activation of serotoninergic neurons, providing further support for the concept of 5-HT mediated inhibition of feeding (Kruk, 1973; Jesperson & Scheel-Kruger, 1973; Blundell, Latham & Lesham, 1973; Clineschmidt, 1973; Samanin, Ghezzi & others, 1972; Funderbunk, Hazelwood & others, 1971; Clineschmidt, McGuffin & Werner, 1974; Ghezzi, Samanin & others, 1973; Garratini, Bizzi & others, 1975). Blundell & Lesham (1975) recently reported that the 5-HT precursor, 5-hydroxytryptophan (5-HTP) has anorectic properties in rats and potentiates the catecholaminergic mediated (Cole & Gay, 1974) anorectic effects of amphetamine, but not the serotoninergically mediated anorectic effects of fenfluramine. These results led the authors to propose that there exists "An inhibitory serotoninergic system for feeding activated by serotoninergic agonists." Whilst this conclusion is in accord with the results of the studies considered above, there is little evidence that the reported anorectic effects of intraventricularly injected 5-HT and peripherally administered 5-HTP are mediated directly by serotoninergic systems rather than by some non-specific disruption of the neurochemical systems controlling food intake. Peripheral administration of 5-HTP results in the formation of 5-HT in many areas of the brain due to the widespread occurrence of the enzyme L-amino acid decarboxylase. It has been shown that 5-HTP may displace catecholamines from intraneuronal granules of catecholaminergic neurons (Fuxe, Butcher & Engel, 1971). Since catecholamines are universally acknowledged to be involved in the control of feeding, it is conceivable that the reported anorectic effects of 5-HTP are due to a general disruption of control of food intake, and that they do not directly involve 5-HT neurons.

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The work reported here was designed specifically to investigate the involvement of serotoninergic neurons in 5-HTP induced anorexia, utilizing a recently discovered inhibitor of the uptake pump in 5-HT neurons, Lilly 110140, 3-(*p*-trifluoromethylphenoxy)-*N*-methyl-3-phenylpropylamine hydrochloride (Wong, Horng & others, 1974).

If the anorectic effects of 5-HTP are mediated by serotoninergic neurons, it should be possible to potentiate them by inhibition of the 5-HT uptake pump, since re-uptake of released 5-HT is the major means of terminating its action at the postsynaptic receptor. It has been reported (Fuller, Perry & others, 1974a) that Lilly 110140 is more potent and more selective in inhibiting the 5-HT uptake pump than chlorimipramine, previously the most selective known uptake inhibitor.

Reports that Lilly 110140 selectively increases concentrations of 5-HT in the hypothalamus (Fuller, Perry & Molloy, 1974b), an area of the brain involved in regulation of food intake and in the anorectic effects of 5-HTP (Blundell & Lesham, 1975) suggested that a test for a specific 5-HT system mediating satiety could be achieved by investigating the effects of Lilly 110140 on food intake and on 5-HTP-induced anorexia.

Male albino rats (University of Liverpool, Psychology Department), 350–500 g at the start of the experiment, were housed individually at 70 (±2) °F in a 12 h light/dark cycle. After three days habituation to their home cages, they were placed on an 18 h deprivation schedule, food being available from 10·30–16·30. Adaptation to this schedule allowed body weights to stabilize after nine days, during which the rats were handled daily. They also received sham injections of saline from the fifth day onwards to minimize the disruptive effects of the injection procedure. On the tenth day rats were weighed and injected with saline 30 min before access to food, and the amount consumed by each rat over the 6 h period was recorded by weighing food (standard laboratory chow) to the nearest 0·1 g. Subsequently, rats were allocated to one of the four groups shown in Table 1, the groups being made up so as to be matched approximately for mean and variance of food intake, as shown.

On the following day (Day 11) the relevant 'pretreatment' was administered at 09·00, followed 1 h later by the relevant 'treatment' (see Table 1). Lilly 110140 was administered as a solution in saline at a dose of 10 mg kg^{-1}. L-5-Hydroxytryptophan (Sigma Chemical Ltd.) was administered as a suspension in saline and Tween 80 at a dose of 30 mg kg^{-1}. All injections were made at a volume equal to 2 ml kg^{-1} (i.p.). The dose of Lilly 110140 was chosen on the basis of reports of its neurochemical effects (Fuller & others, 1974b), and the dose of L-5-HTP on the basis of reports of its anorectic potency (Blundell & Lesham, 1975). Thirty min after 'treatment' (10·30), rats received access to standard laboratory chow and the amounts eaten by each rat 1, 2, 4 and 6 h later were recorded by weighing food to 0·1 g. The mean (±s.e.) cumulative amounts of food eaten at each sampling time for each group are shown in Table 1.

A preliminary analysis of these data by a Two-Way ANOVA for repeated measures (using the amounts eaten expressed as rates per h between each sampling time), indicated that there were highly significant main effects of groups ($F = 15{\cdot}90$; df = 3,114; $P < 0{\cdot}001$) and of sampling times ($F = 16{\cdot}59$; df = 3,114; $P < 0{\cdot}001$), as well as a highly significant interaction ($F = 6{\cdot}94$; df = 9114; $P < 0{\cdot}001$). Individual comparisons between groups were undertaken by two-tailed *t*-tests on the cumulative amounts eaten at each sampling time, as shown in Table 1. 5-HTP had a potent anorectic effect ($P < 0{\cdot}01$ at all times), as did the combination of Lilly 110140 and 5-HTP ($P < 0{\cdot}001$). Lilly 110140 was found to have an anorectic effect of short duration (≏ 2 h). Six h after access to food the cumulative amount eaten by subjects pretreated with Lilly 11040 alone did not differ significantly from controls ($t = 0{\cdot}77$, $P > 0{\cdot}2$), or from the amount eaten by the same subjects over 6 h on the previous day ($t = 0{\cdot}79$, $P > 0{\cdot}2$). However, Lilly 110140 potentiated the anorectic effect of 5-HTP ($P < 0{\cdot}01$ at 1, 2 and 4 h) at all sampling times, even after the anorectic effect of the drug itself had completely worn off ($P < 0{\cdot}001$ at 6 h).

Between 2 and 6 h after food access, rats in the Lilly 110140 group ate at a faster rate than controls. The short duration anorectic effects observed at 1 and 2 h after food access were consequently compensated for in the last 4 h of food access when Lilly 110140 did not affect food intake at all.

These data indicate not only that Lilly 110140 has a potent anorectic effect of short duration, but also that it potentiates the anorectic effects of 5-HTP. Significantly, this potentiation was observed even after the

Table 1. *Details of experimental groups.*

Group	Pretreatment	Treatment	Mean (±s.e.) food intake in 6 h on Day 10	Sampling time† 1 h	2 h	4 h	6 h
Controls	Saline	Saline	17·99 ± 2·93	8·12 ± 0·68	10·24 ± 1·14	12·69 ± 1·37	17·84 ± 0·86
5-HTP	Saline	5-HTP	18·93 ± 1·63	3·76 ± 0·71**	5·24 ± 0·74**	8·74 ± 1·56**	12·29 ± 1·89**
Lilly 110140	110140	Saline	17·93 ± 1·33	3·77 ± 0·75**	6·54 ± 1·00*	9·90 ± 0·85	16·63 ± 1·49
Lilly 110140-5-HTP	110140	5-HTP	19·65 ± 1·25	0·67 ± 0·28***	1·49 ± 0·59***	3·82 ± 0·96***	5·31 ± 0·86***

† Mean cumulative amounts (±s.e.) eaten on Day 11 in g by subjects in each group at each sampling time. Significant differences from controls (Two tailed *t*-tests):— * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

anorectic effect of Lilly 110140 had completely worn off (between 2 and 6 h after food access), indicating that the potention of 5-HTP anorectic effects observed in the Lilly 110140–5-HTP group is not solely due to synergic anorectic effects of Lilly 110140 and 5-HTP but is also a consequence of specific inhibition of the 5-HT uptake pump. The results consequently *directly* implicate serotoninergic neurons in the anorectic effects of 5-HTP.

The observed transient anorectic effects of Lilly 110140 are also compatible with the notion that a serotoninergic system inhibits feeding since Lilly 110140 has been reported to elevate hypothalamic concentrations of 5-HT without affecting concentrations in other areas of the brain (Fuller & others, 1974b). A possible explanation for the short duration of the anorectic effect is that overstimulation of the postsynaptic receptor causes a compensatory decrease in 5-HT turnover. It would seem unlikely that the short duration of anorectic action can be explained in terms of the drug having a short half life since it has been reported that Lilly 110140 has a very long (24–48 h) duration of action in inhibiting the uptake pump (Wong & others, 1975; Fuller & others, 1975). In support of the suggestion that the drug's short duration of anorectic action is due to a compensatory decrease in 5-HT turnover is the finding that tricyclic antidepressants which inhibit the 5-HT uptake pump slow the firing rates of serotoninergic neurons in the midbrain raphé nuclei (Bramwell, 1972). Furthermore, Lilly 110140 itself has been shown to significantly decrease 5-HT turnover in rats 4 h after administration at the dose used in this study (Fuller & others, 1974b). The transient anorectic effects of the uptake inhibitor are consequently compatible with the concept of a serotoninergic system which inhibits food intake, although an adequate explanation of the drug's anorectic effects awaits a more definitive neurochemical study.

These results are not merely a reflection of potentiation of the sedative effects of 5-HTP. Such potentiation might conceivably result in a gross degree of incapacitation, rendering rats incapable of eating. Although in both the 5-HTP and the Lilly 110140—5-HTP groups the rats appeared sedated during the period of food access, there were no overt differences between the two groups. The animals in the Lilly 110140—5HTP group showed normal placing and righting reflexes, and there were no overt signs of toxicity due to the drug combination. Jacobs & Ewbank (1974) and Modigh (1974) have shown that the sedative effects of 5-HTP are peripherally mediated, which would appear to rule out this interpretation of the results since the demonstration that 5-HTP has differential anorectic potency in rats with lesions of the lateral hypothalamus and controls (Blundell & Lesham, 1975) suggests that the anorectic effects of 5-HTP are centrally mediated. The data we report indicated that such effects are mediated by 5-HT neurons.

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