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The effect of 5-hydroxytryptophan on food intake and on the anorexic action of amphetamine and fenfluramine

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5-Hydroxytryptophan (5-HTP), amphetamine and fenfluramine suppressed food intake in normal rats and in animals with lesions of the lateral hypothalamus. The anorexic effect of amphetamine was reduced in lesioned animals compared with controls while the effect of 5-HTP like that of fenfluramine was increased. When administered in conjunction with the anorexic drugs, 5-HTP markedly potentiated the anorexic effect of amphetamine in both control and lesioned animals. However, 5-HTP potentiated fenfluramine anorexia only in lesioned rats. These findings provide further evidence for the role of 5-hydroxytryptamine (5-HT), in the anorexic effect of fenfluramine, and suggest that a 5-HT mechanism, inhibitory for feeding, produces particularly severe suppression of food intake in rats with lateral hypothalamic lesions.

Since fenfluramine has been shown to exert a selective action on brain 5-hydroxytryptamine (5-HT) concentrations (e.g. Duhault & Verdavainne, 1967; Morgan, Cattabeni & Costa, 1972) it has been suggested that many of the behavioural changes induced by fenfluramine depend upon alterations in 5-HT metabolism. For example, it has been shown that injections of 5-hydroxytryptophan (5-HTP), the precursor of 5-HT, mimics the effect of fenfluramine on temperature (Jesperson & Scheel-Krüger, 1970), sleep (Zolovick, Stern & others, 1973) and a simple conditioned response (Southgate, Mayer & others, 1971).

For feeding, it has been reported that 5-HT blocking drugs such as methergoline (Funderburk, Hazelwood & others, 1971; Jesperson & Scheel-Krüger, 1973) or methysergide (Blundell, Latham & Leshem, 1973) antagonize the anorexic effect of fenfluramine but not of amphetamine, while lesions of the midbrain raphe nuclei, which selectively deplete the brain of 5-HT, abolish fenfluramine anorexia but leave amphetamine anorexia unaltered (Samanin, Ghezzi & others, 1972). In addition, injections of cyproheptadine have been reported to counteract the anorexic effect of intraventricular injections of 5-HT and fenfluramine (Kruk, 1973) and Clineschmidt (1973) has shown that fenfluramine anorexia is partially inhibited by 5, 6-dihydroxytryptamine a compound believed to bring about selective degeneration of central 5-HT containing neurons (e.g. Baumgarten & Lachenmayer, 1972). It seems that fenfluramine may release 5-HT from the presynaptic terminal and also prevent its reuptake (Garattini, Buczko & others, 1974).

Unlike fenfluramine, the anorexic effect of amphetamine seems mainly to depend upon a catecholaminergic rather than a serotonergic system (Holtzman & Jewett, 1971; Ahlskog & Hoebel, 1973). In addition, since quite different anorexic effects have been observed following intrahypothalamic injections, these two drugs appear to operate through separate central sites of action (Blundell & Leshem, 1973). This

belief was confirmed by a recent study showing that lesions of the lateral hypothalamus (LH) the site of the classical feeding centre (Anand & Brobeck, 1951), exerted a distinctive influence on the anorexic potency of each of the two drugs: amphetamine anorexia was ameliorated while fenfluramine anorexia was enhanced (Blundell & Leshem, 1974). The increased severity of the appetite suppressant effect of fenfluramine in animals with LH lesions was believed to be due to an enhanced inhibitory action of a serotonergic mechanism. Accordingly, the present experiment was designed to further investigate this phenomenon by comparing the effects of 5-HTP and fenfluramine upon feeding in normal animals and in rats with lesions of the LH. In addition the experiment investigated the interaction of 5-HTP with amphetamine and fenfluramine.

MATERIALS AND METHODS

Subjects and surgery

Lesions were produced electrolytically in male hooded rats (280–350g at the time of operation) under ether anaesthesia by passing a 1 mA current through a 0·5 mm diameter anode and a rectal cathode for 10 s. The uninsulated 0·5 mm tip of the electrode was stereotaxically positioned at co-ordinates A5·4–5·0, $\pm$ 1·5, 8·8 deep (De Groot, 1959). Animals received unilateral lateral hypothalamic lesions (ULH), half of the lesions being placed on the right and half on the left side of the brain; bilateral lesions (BLH); or control lesions (C). In some control animals an electrode was lowered to both sides of the hypothalamus but no current was passed, while other animals simply had burrholes placed in the skull with no penetration of brain tissue.

At the conclusion of the experiment the position and extent of the hypothalamic lesions were determined from photographic enlargements of unstained 50 μm frozen sections.

Procedure and design

After surgery, the lesioned animals received special nutritional care to counteract the period of aphagia and adipsia which follows lateral hypothalamic destruction. Rats that refused dry food pellets were given sweet mash and where necessary they were fed intragastrically twice daily. Animals were gradually weaned from intragastric feeding and consumption of sweet mash and were fed on dry pellets for at least 2 weeks before drug injections were given.

The experiment was begun three months after surgery and during the feeding tests food was removed from the animals at 18·00 h in the evening and at 10·00 the following day a weighed amount of food was placed in the cages. Food intake was monitored periodically over 24 h and measurements were taken after 1, 4, 8 and 24 h. Spillage was collected on tissue paper placed beneath the wire mesh cage floor and weighings were made to the nearest 0·1 g.

Three procedures were adopted to ensure minimal disturbance of the animals during the drug injection series. After surgical treatment all animals were rendered docile by being handled daily when removed from their cages for weighing. In addition, the food deprivation regime was initiated 10 days before the start of chemical injections to allow time for animals to stabilize their feeding pattern, and for 7 days before drug administration animals received sham intraperitoneal injections to allow habituation to the stress of the injection procedure before the start of the experiment proper.

At least 72 h intervened between testing sessions and on every test day each animal received two injections. At 40 min before the beginning of the feeding test the animals were given intraperitoneal injections (0·5 ml volume) of either 37·5 mg kg^{-1} L–5-hydroxytryptophan or 0·9% w/v saline. This dose of 5-HTP has been reported to reverse deficits brought about by low brain 5-HT (Lints & Harvey, 1968) and has been shown to increase the level of brain 5-HT for approximately 3 h after injection (Harvey & Lints, 1971).

Thirty minutes before the food test, the animals received a second injection of 1·0 mg kg^{-1} (+)-amphetamine sulphate, 5·0 mg kg^{-1} (±)-fenfluramine hydrochloride or 0·9% w/v saline. The study therefore conformed to a 3 (lesion groups) by 2 (precursor condition) by 3 (drug condition) factoral design, and over the course of the experiment each animal was given each pair of the six different combinations of injections. The results were analysed by an analysis of variance procedure for repeated measures (Winer, 1970), and 2—sample tests of planned *a priori* comparisons were carried out using Student's *t*-test.

RESULTS

Histology

Inspection of the photographic enlargements of the coronal brain sections indicated that the lateral hypothalamic lesions consistently destroyed an area of the lateral hypothalamus adjacent, and slightly dorsal, to the descending column of the fornix in the De Groot planes A5·4–4·6. The lesions straddled the mid- and far-lateral areas of Morgane (1961) and in some cases encroached upon the medial edge of the internal capsule and the ventral edge of the zona incerta.

Body weight

Fig. 1 shows the effect of the various lesion procedures on body weight. In keeping with the histological evidence, the weight of the bilaterally lesioned animals fell precipitously following operation but began to recover after about one week during the period of extra nutritional care. Six weeks after operation weights had begun to stabilize and it is noticeable that the mean weight of the unilaterally lesioned group falls between that of the bilateral and sham lesioned animals.

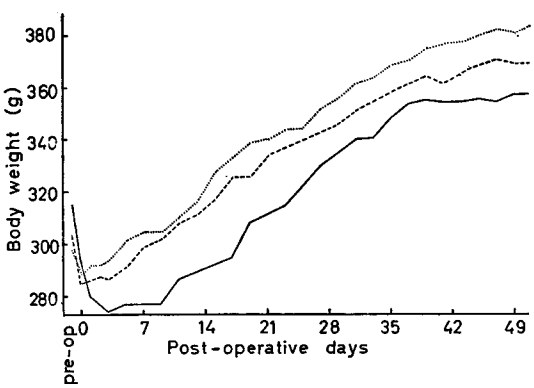

FIG. 1. Changes in body weight following bilateral (BLH; —), unilateral (ULH; - - -) or sham (· · · ·) lesions of the lateral hypothalamus.

5-Hydroxytryptophan and food intake

The analysis of variance showed a significant main effect for the precursor condition ($F = 52{\cdot}28$, d.f. 1 and 22, $P < 0{\cdot}01$) indicating that injections of 5-HTP depressed food intake. Over all conditions, 5-HTP brought about a 29·2% reduction in feeding for the 0–1 h test period. Moreover, the precursor X lesion interaction was significant ($F = 14{\cdot}18$, d.f. 1 and 22, $P < 0{\cdot}01$) showing that the influence of 5-HTP on food intake was modified by the influence of hypothalamic lesions (Fig. 2). Further analysis of this interaction revealed that 5-HTP exerted a more severe suppressive effect on food intake in the BLH animals than in controls ($t = 3{\cdot}1$, d.f. 22, $P < 0{\cdot}01$). Measurements taken at 4, 8 and 24 h after injection showed that 5-HTP had ceased to exert an anorexic action by the 8 h tests. Since ULH animals did not differ markedly from controls, this group was omitted from subsequent statistical analysis.

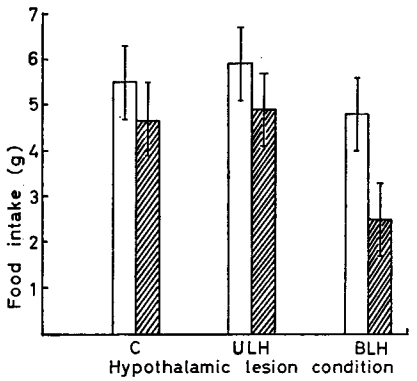

FIG. 2. Effect of injections of (—)-5-hydroxytryptophan on feeding in animals with bilateral (BLH), unilateral (ULH), or sham (C) lesions of the lateral hypothalamus. Histograms indicate mean food intake (g) ± standard error for the 0–1 h feeding period (open columns = saline; hatched colums = 5-HTP). See text for statistical analysis.

Anorexic drugs and food intake

The main effect due to the drug condition was highly significant ($F = 121{\cdot}69$, d.f. 2 and 44, $P < 0{\cdot}01$) indicating that the anorexic drugs reduced food intake. In addition, the drug X lesion interaction was significant ($F = 3{\cdot}68$ d.f. 2 and 44, $P < 0{\cdot}05$) showing that the anorexic action of the drugs was selectively influenced by the lesion condition. Amphetamine brought about a 60% reduction of food intake in the control animals but only a 50% diminution in the BLH group. On the other hand, fenfluramine gave rise to a 45% suppression in controls but induced a 54% reduction in lesioned rats. These results, though less impressive than those obtained in a previous study (Blundell & Leshem, 1974), clearly confirm that amphetamine anorexia is ameliorated by lateral hypothalamic lesions while fenfluramine anorexia is enhanced.

Interaction between 5-HTP, anorexic drugs, and hypothalamic lesions

The precursor X drug interaction just failed to reach the 5% level of significance ($F = 3{\cdot}19$, df 2 and 44, P approx. 0·05) but strongly suggested that 5-HTP was exerting a selective effect on the action of the anorexic drugs. This was confirmed

by a closer inspection of the data. It was found that 5-HTP enhanced the anorexic potency of both anorexic drugs but the enhancement was greater for amphetamine ($t = 4{\cdot}15$, d.f. $= 46$, $P < 0{\cdot}01$) than for fenfluramine ($t = 1{\cdot}95$, d.f. $= 46$, $P < 0{\cdot}1$, 2-tailed tests). The reason for the more marked facilitation of amphetamine anorexia became apparent when the effect of 5-HTP on amphetamine and fenfluramine was examined separately for the BLH group and the control animals (Table 1). In the BLH group 5-HTP caused a significant potentiation of the anorexic effect of both

Table 1. *Effect of 5-HTP on the anorexic action of amphetamine and fenfluramine in BLH and C animals.* Figures shown are means and standard errors for food intake (g). *Indicates 5-HTP significantly different from saline (smallest $t = 2.22$, df 22, $P < 0{\cdot}05$). In control animals 5-HTP fails to potentiate the anorexic effect of fenfluramine ($t = 0{\cdot}25$, df 22, $P < 0{\cdot}2$).

Precursor injection	Lesion group			
	BLH		C	
	Anorexic drug			
	Amphetamine	Fenfluramine	Amphetamine	Fenfluramine
Saline	3·7 ± 0·65	3·5 ± 0·55	3·5 ± 0·55	4·5 ± 0·53
5-HTP	0·9* ± 0·38	1·0* + 0·19	1·9* ± 0·37	4·3 ± 0·60

amphetamine and fenfluramine. However, in the control lesion group, 5-HTP exerted a marked enhancement of amphetamine anorexia but failed to modify fenfluramine anorexia. This strong facilitatory effect of 5-HTP on amphetamine anorexia can be contrasted with the strong antagonistic effect of 5-HTP on amphetamine induced stereotypy (Weiner, Goetz & others, 1973) and a weak antagonistic effect on amphetamine hyperactivity (Mabry & Campbell, 1973).

DISCUSSION

The results of this experiment show that injections of 5-HTP give rise to a significant anorexic effect in deprived rats. This finding confirms a previous study which reported a small reduction in food intake following administration of 5-HTP to satiated rats (Joyce & Mrosovsky, 1964). Moreover, in the present experiment the anorexic action of 5-HTP was mild in normal animals but considerably enhanced in BLH rats. This pattern of anorexic potency is similar to that of fenfluramine but the opposite of that brought about by amphetamine, and these findings suggest that 5-HTP and fenfluramine may bring about anorexia by means of the same or similar mechanisms. Since the anorexic effect of fenfluramine is believed to depend on direct (Funderburk & others, 1971), or indirect (Garattini & others, 1974) stimulation of serotonergic neurons, and since 5-HTP administration leads to a rapid increase in brain 5-HT (Udenfriend, Weissbach & Bogdanski, 1957; Harvey & Lints, 1971), it seems likely that both chemicals activate a serotonergic system which mediates inhibition of feeding. This proposal must be considered in the light of the report of Moir & Eccleston (1968) that 5-HTP administration leads to the formation of brain 5-HT in non-serotonergic systems. Hence the effect upon food intake of drugs altering 5-HT metabolism may be brought about by a non-specific blockade of feeding rather than by intervention in a specialized serotonergic system inhibitory for feeding.

However, the observation that 5-HTP and fenfluramine exert a similar influence on feeding behaviour is in accord with previous reports indicating that 5-HTP mimics the effect of fenfluramine on temperature, sleep, stereotyped behaviour and one-trial conditioning, and provides further evidence for the belief that serotonergic mechanisms are widely implicated in the behavioural effects of fenfluramine. In addition, it is noticeable that fenfluramine, like 5-HTP, antagonizes amphetamine induced stereotypy (Garattini, Buczko & others, 1974) but enhances amphetamine anorexia (Leshem & Blundell, 1974).

Moreover, the notion of an inhibitory serotonergic system for feeding activated by serotonergic agonists provides an explanation for the enhancement of amphetamine anorexia by 5-HTP for these drugs appear to induce anorexia by means of separate mechanisms: amphetamine seems to act upon the terminals of the ventral noradrenergic bundle which participates in the suppression of feeding (Ahlskog & Hoebel, 1973), while 5-HTP activates an inhibitory serotonergic system. Hence it would be expected that the anorexic effects of each drug would be additive. In contrast, since it has been suggested that the anorexic actions of 5-HTP and fenfluramine are mediated by the same serotonergic system their effects would not be expected to summate. This supposition was confirmed in sham lesioned animals (group C) where the anorexic effects of 5-HTP and fenfluramine were not additive. However, in the BLH group 5-HTP markedly enhanced the anorexic potency of fenfluramine, a finding which runs counter to this supposition but which is consistent with the previously noted increased severity of anorexia of both 5-HTP and fenfluramine in BLH animals. This constellation of findings seems to indicate that the activation of a serotonergic system (by 5-HTP, fenfluramine, or both drugs acting simultaneously) gives rise to an abnormally severe suppression of feeding in animals with lateral hypothalamic lesions. One possible explanation is that the lesions interrupt ascending 5-HT axons from the raphe nuclei coursing upwards through the hypothalamus (Dahlstrom & Fuxe, 1965); in turn this could lead to the supersensitivity of postsynaptic 5-HT receptors in the same way that lesions of the dopaminergic nigrostriatal system lead to receptor hypersensitivity to apomorphine and laevodopa (Ungerstedt, 1971). Alternatively, since it is known that lesions of the medial forebrain bundle in the area of the lateral hypothalamus give rise to marked alterations in brain amine concentrations (Oltmans & Harvey, 1972), it is possible that the enhancement of 5-HTP and fenfluramine anorexia in the BLH group was due to an impairment of hunger motivation (Dicara & Wolf, 1968) in animals with reduced levels of brain catecholamines responsible for the facilitation of feeding (e.g. Berger, Wise & Stein, 1971).

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