Failure of depletion of rat brain 5-hydroxytryptamine to alter fenfluramine-induced anorexia

It has been proposed that fenfluramine-induced anorexia may depend upon a direct or indirect effect on central 5-hydroxytryptamine (5-HT) mechanisms (Jespersen & Scheel-Kruger, 1970, 1973; Southgate, Mayer & others, 1971; Funderburk, Hazelwood & others, 1971; Samanin, Ghezzi & others, 1972; Blundell, Latham & Leshem, 1973; Ghezzi, Samanin & others, 1973; Kruk, 1973; Blundell & Leshem, 1975; Buczko, De Gaetano & Garattini, 1975). Evidence for an indirect mechanism of action is the fact that the anorectic effect of fenfluramine is antagonized in rats selectively depleted of brain 5-HT either by midbrain raphé lesioning (Samanin & others, 1972) or by the intraventricular injection of 5,6-dihydroxytryptamine (Clineschmidt, 1973). Clineschmidt, McGuffin & Werner (1974) have recently proposed that the anorectic effect of large doses of fenfluramine (5–8 mg kg^{-1}, i.p.) depends upon the release of 5-HT from central serotoninergic neurons. On the other hand, anorexia induced by doses of fenfluramine less than 6 mg kg^{-1} is not dependent upon a 5-HT mediated mechanism of action. Should this hypothesis be correct, procedures which deplete the brain of 5-HT should leave unaltered the anorectic effect of a low dose of fenfluramine, whilst antagonizing anorexia induced by a large dose of the drug. To test this hypothesis the anorectic effect of fenfluramine was examined in rats submitted to three procedures to selectively lower rat brain 5-HT concentrations; by electrolytic lesions of the midbrain raphé an area rich in 5-HT containing cell bodies (Kostowski, Giacalone & others, 1968), by the intraventricular injection of 5,6-dihydroxytryptamine to cause selective degeneration of central 5-HT containing neurons (Baumgarten, Bjorklund & others, 1971) and by the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine (Koe & Weissman, 1966).

Male Wistar rats, 160–190 g at the beginning of the experiment were used. A group of pentobarbitone sodium anaesthetized rats was lesioned in the midbrain raphé using a Radionics radio frequency generator (55° for 1 min) through a Radionics 0·7 mm diameter probe uninsulated 1·5 mm from the tip implanted stereotaxically according to the following co-ordinates: A = 0·4; L = 0; D = −2·6 (Konig & Klippel, 1963). Control rats were similarly operated but not lesioned. Other rats were anaesthetized with halothane and 5,6-dihydroxytryptamine creatinine sulphate monohydrate (Regis Chem. Co.) (100 μg in 20 μl) was infused over 1 min into the right lateral ventricle of the brain (Noble, Wurtman & Axelrod, 1967). The drug was freshly prepared in 0·9% sodium chloride containing ascorbic acid (1 mg ml^{-1}). Controls were treated in a similar manner except that 5,6-dihydroxytryptamine vehicle was infused. Three days after midbrain raphé lesioning or intraventricular 5,6-dihydroxytryptamine, rats, together with their appropriate controls, were individually caged and conditioned to eat over a 3 h period (11·00–14·00) (water *ad lib*). Food was standard rat diet in a powdered form and intake was measured daily. Intake was essentially stabilized after one week. After 14 days on the feeding schedule (±)-fenfluramine hydrochloride (generously donated by Servier Laboratories Limited) was injected (i.p.) 30 min before exposure to food. In all experiments the mean food intake of each rat for the three days before fenfluramine administration was calculated and the food intake of each rat following drug administration was expressed as a percentage of this mean value. Other rats were injected with *p*-chlorophenylalanine methylester hydrochloride (AB Biotec) (100 mg kg^{-1}, i.p.) once daily at 10.00 h on days 11, 12 and 13 of the eating schedule. Controls received a similar volume of 0·9% sodium chloride. Further groups of rats were subjected to identical experimental procedures to those described above except that, instead of receiving fenfluramine on day 14 of the eating schedule, they were killed by a sharp

Table 1. *Effect of midbrain raphé lesioning (ML), intraventricular 5,6-dihydroxytryptamine (DHT) and p-chlorophenylalanine pretreatment (PCPA) on fenfluramine-induced anorexia in the rat.* Control food intake is the mean daily food intake of each rat for the 3 days before drug administration. Fenfluramine was injected i.p. 30 min before exposure to food. For further experimental information see text.

Pretreatment	Fenfluramine (mg kg^{-1})	n	Control food intake (g ± s.e.m.)	Test day as % of control (Mean ± s.e.m.)
None	4	8	15·42 ± 0·74	54·90 ± 4·78
ML	4	9	14·33 ± 0·33	57·17 ± 1·94
DHT	4	9	15·31 ± 0·55	44·96 ± 3·64
PCPA	4	10	11·76 ± 0·37***	48·80 ± 5·36
None	8	8	14·42 ± 0·65	29·56 ± 2·75
ML	8	7	14·84 ± 0·79	22·10 ± 3·38
DHT	8	7	14·31 ± 0·58	21·27 ± 4·68
PCPA	8	8	12·42 ± 0·41*	21·76 ± 2·84

* $P < 0{\cdot}05$.
*** $P < 0{\cdot}001$.

blow on the head and the brain quickly removed. Following extraction (Neff & Costa, 1966), tissue concentrations of noradrenaline and dopamine were assayed spectrophotofluorometrically (Laverty & Taylor, 1968). Brain 5-HT was extracted and assayed by the method of Snyder, Axelrod & Zweig (1965). Amine concentrations were calculated as $\mu g\ g^{-1}$ of wet tissue, results being corrected for 100% recovery. In all experiments doses refer to the free base.

Of the three 5-HT-depleting procedures employed, only *p*-chlorophenylalanine pretreatment significantly altered rat food intake (Table 1). Furthermore, the anorectic effect of both doses of fenfluramine (4 and 8 mg kg^{-1}) was unaltered either by midbrain raphé lesioning or by intraventricular 5,6-dihydroxytryptamine or by *p*-chlorophenylalanine pretreatment (Table 1). Although not shown in Table 1 the food intake of rats which were either sham lesioned or had received 5,6-dihydroxytryptamine vehicle intraventricularly did not differ from that of untreated rats.

The failure of *p*-chlorophenylalanine pretreatment to modify the anorectic effect of fenfluramine is in agreement with the findings of others (Funderburk & others, 1971; Clineschmidt, 1973). However, the failure of both midbrain raphé lesioning and intraventricular 5,6-dihydroxytryptamine to alter the fenfluramine-induced inhibition of rat food intake contrasts with the findings of Samanin & others (1972) and Clineschmidt (1973).

The effect of the three procedures on rat brain concentrations of noradrenaline, dopamine and 5-HT is shown in Table 2. Only rat brain 5-HT content was significantly altered by the three procedures. The reduction in rat brain 5-HT following midbrain raphé lesioning observed in this study (−64·5%) is in good agreement with the findings (−67%) of Samanin & others (1972). Similarly, the 5,6-dihydroxytryptamine-induced fall in brain 5-HT content observed (26·7%) agrees with the data (−35%) of Clineschmidt (1973). Hence the discrepancy in findings cannot be attributed to differences in the degree of brain 5-HT depletion achieved.

As to why both midbrain raphé lesioning and intraventricularly injected 5,6-dihydroxytryptamine lead to contradictory results remains to be elucidated. The use of both intraventricular 5,6-dihydroxytryptamine and midbrain raphé lesioning to

Table 2. *Effect of midbrain raphé lesioning (ML), intraventricular 5,6-dihydroxytryptamine (DHT) and* p*-chlorophenylalanine pretreatment (PCPA) on rat brain noradrenaline (NA), dopamine (DA) and 5-HT concentrations.* Results are expressed as % of appropriately treated controls. For further experimental information see text.

Treatment	n	Endogenous amine content as % of control (Mean ± s.e.m.) NA	DA	5-HT
ML	10	110·4 ± 3·5	107·3 ± 8·9	35·6 ± 0·9*
DHT	10	96·9 ± 3·4	95·8 ± 6·0	73·3 ± 1·0*
PCPA	6	95·8 ± 3·7	98·6 ± 6·0	45·6 ± 2·5*

* $P < 0{\cdot}001$.

lower rat brain 5-HT content has resulted in other contradictory results. For example, morphine analgesia in rats has been reported to be antagonized both by midbrain raphé lesioning (Samanin, Gumulka & Valzelli, 1970) and by intraventricular 5,6-dihydroxytryptamine (Genovese, Zonta & Mantegazza, 1973). On the other hand, Blasig, Reinhold & Herz (1973) found that both procedures did not alter morphine analgesia. Perhaps it could be argued that there may be some strict localization of a specific pathway and that the interruption of such a pathway may be the critical determinant. As an extension of this concept it could be hypothesized that the approaches used to lower brain 5-HT both in this and in the other studies cited above are not sufficiently specific and also suffer from the inherent weakness in assuming that whole brain 5-HT acts in a unitary manner to produce biological responses. However, recent studies show that this is not so. For example, lesions of the nucleus raphé medianus and the nucleus raphé dorsalis produce approximately equal falls in rat brain 5-HT content yet only median lesions increase locomotor activity (Jacobs, Wise & Taylor, 1974) and antagonize morphine analgesia (Adler, Kostowski & others, 1975).

The results of this study suggest that the anorectic effect of both high and low doses of fenfluramine is not mediated via an indirect effect on rat brain 5-HT systems. Whilst the possibility should not be excluded that fenfluramine-induced anorexia may be due to an indirect effect upon an, as yet unidentified, specific brain 5-HT system, the concept of the drug acting directly on specific central 5-HT receptors regulating food intake warrants consideration.

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June 23, 1975

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Influence of the adrenals and gonads on the plasma kininogen concentrations in male and female rats

A relation has been established between the concentration of reproductive hormones and the plasma kininogen concentrations in the rat. Previous work has shown that oestrogens will raise plasma kininogen concentration in the rat and progesterone will lower the kininogen concentration in either intact or ovariectomized female rats (McCormick & Senior, 1974; Senior & Whalley, 1974). In the male rat a relation has been established between the plasma kininogen concentration and treatment with androgens. If androgens are administered to the male or female then the plasma kininogen concentration is lowered in intact rats (McCormick & Senior, 1974). The actual mechanism by which these factors influence plasma kininogen values has not yet been elucidated but the evidence so far suggests that reproductive hormones are mediators in the normal maintenance of the kininogen concentrations. Other workers have noted that following adrenalectomy in the rat there is an increase in plasma kininogen concentration (Rosa, Rothschild & Rothschild, 1972). This increase following adrenalectomy could result from a change in the reproductive hormone concentrations and the work reported here was undertaken to investigate the interrelation between the gonads and adrenals in the regulation of plasma kininogen concentrations.

The work was divided into two sections, one involving female and the other male rats. The animals used all weighed 200–250 g and were housed in groups of six in light- and temperature-controlled conditions. They were allowed free access to food and were all given normal saline to drink from the day of operation. Adrenalectomy