J. Pharm. Pharmac., 1976, **28**, 471–477 *Received February 5, 1976*

Differences between the anorexic actions of amphetamine and fenfluramine—possible effects on hunger and satiety*

J. E. BLUNDELL†, C. J. LATHAM AND M. B. LESHEM

Psychology Department, University of Leeds, Leeds LS2 9JT, U.K.

The inhibition of feeding in rats brought about by amphetamine and fenfluramine was continuously monitored for periods of up to 24 h using a pellet detecting eatometer. For rats tested under conditions of food deprivation the two drugs gave rise to distinctive anorexic profiles: amphetamine delayed the onset of eating whereas fenfluramine allowed eating to commence normally but brought about an early termination of the initial bout of feeding. When the drugs were administered to rats with free access to food, analysis of the meal pattern showed that amphetamine gave rise to a small increase in the inter-meal interval while fenfluramine brought about a clear reduction in meal size. It is suggested that the contrasting modes of action of these drugs represent an effect of amphetamine upon hunger and an action of fenfluramine on satiety. This suggestion is in keeping with the proposed mechanisms of action of these drugs, amphetamine acting upon a hypothalamic motivational system and fenfluramine acting by means of a postulated serotoninergic satiety system. Use of the continuous monitoring technique has pointed to certain limitations in the assessment of anorexic drug action by means of discrete food sampling periods.

In many studies on the action of anorexic drugs, the effect of the drug is assessed by its capacity to reduce the total amount of food consumed in a given time, varying from 15 min to hours, and in certain cases animals are trained to consume their daily intake in 4, 6 or 8 h. These procedures provide information about the quantitative effects of anorexic drugs on food intake and the effectiveness of different drugs may then be compared using the traditional ED50 measure or an RD50 value (Cox & Maickel, 1972).

However, measurement of the bulk of food consumed during brief periods overlooks qualitative aspects of the feeding process and consequently provides only a crude technique for comparing the actions of different drugs. Firstly, following a period of deprivation animals vary the rate of their food consumption over time; since the time courses of action of different drugs may also vary (e.g. Blundell, Campbell & others, 1975), a discrete observation period for monitoring the maximal effect of one drug may neglect important information about another. Secondly, drugs may exert their anorexic action through different components of the systems regulating patterns of feeding (Blundell, 1975a), and simple measurement of weight of consumed material will fail to distinguish between these modes of action.

We have used a technique which permits the continuous monitoring of feeding over long periods and the precise measurement of food intake over short intervals. The procedure involves the use of a pellet detecting eatometer (Kissileff, 1970) which delivers a small (45 mg) food pellet to replace one eaten by the animal and provides a means of recording every single pellet consumed during the experiment. When animals are tested following a period of food deprivation the technique provides a way of distinguishing between drugs which may postpone the onset of eating, slow the rate of consumption, or alter the length of the initial bout of feeding. For animals with free access to food the action of drugs on meal patterns can be assessed by an effect upon meal frequency, meal size, inter-meal intervals and the relationships between these indices. An examination of these qualitative aspects of feeding allows the possibility of discriminating between drugs which may act primarily upon hunger, or upon satiety. Accordingly, the following series of experiments has investigated certain qualitative features of the effect on feeding of two anorexic drugs—amphetamine and fenfluramine—which have been shown to display contrasting pharmacological profiles (e.g. Garattini, 1972).

* A brief account of this work was presented at the 1st International Congress on Obesity, London 1974.

† Correspondence.

MATERIALS AND METHODS (EXP. I)

Apparatus

The eatometer was a V-shaped brass trough with a light source embedded in one end so that a light beam was directed along the base of the V on to a photocell. When a 45 mg food pellet rested in the trough the light beam was broken, but removal of the pellet activated the photocell and triggered the delivery of a further pellet from a Gerbrandt's pellet dispenser. Every pellet delivered was automatically counted and recorded and a pellet was always available in the eatometer. The eatometer itself was fitted to the side of the cage, and each cage was enclosed in a sound-attenuating chamber which was programmed with a 12 h light-dark cycle.

Design and procedure

Male black-hooded rats, 320–380 g, were placed in individual cages and allowed two weeks to become accustomed to obtaining their food supply from the eatometer. The rats were then subjected to a cyclical deprivation schedule. For this procedure the eatometers were inactivated at 6 o'clock in the evening and switched on again at 10 o'clock the following morning. Each 16-h deprivation period encompassed the 12 h dark portion of the light-dark cycle and was followed by a period of free access to food for 32 h. The cycle was then initiated once more and was continued for a further two weeks to allow the rats to habituate to the deprivation schedule. In addition, during this period the rats were administered sham intraperitoneal injections to accustom them to the stress of the injection procedure before the start of the experiment proper. These sham injections were given at the end of each deprivation period when the animals were removed from their cages for weighing.

The experiment was conducted in two phases. In the first phase the rats were injected (i.p.) with doses of (+)-amphetamine sulphate (1·75 mg kg^{-1}) and (±)-fenfluramine hydrochloride (3·75 mg kg^{-1}) which we had found to be the approximate ED50 values of these drugs for a 2 h food intake period following 16 h of food deprivation. Injections of amphetamine, fenfluramine and 0·9% w/v saline were given to each of four rats using a counterbalanced order of drug presentation to minimize temporal order effects. Each rat received each drug twice and at least 48 h intervened between injections. In the second phase of the experiment the same rats were used to establish dose-response relations of the temporal anorexic profiles of amphetamine and fenfluramine, and to confirm that the pattern of food intake suppression observed in phase one was displayed with differing doses of the drugs. Accordingly, three doses of amphetamine (1·0, 2·5 and 5·0 mg kg^{-1}) and of fenfluramine (1·0, 2·5 and 5·0 mg kg^{-1}) were administered to the rats in a counterbalanced order with each rat receiving a single injection of each drug. In both phases of the experiment, all injections were made 30 min before the eatometers were switched on, at which time every pellet consumed by the rats was continuously monitored for at least 8 h and in some cases for a full 24 h.

RESULTS AND DISCUSSION (Exp. I)

Fig. 1 shows the results from phase one of the experiment, and observation of the temporal profiles of amphetamine and fenfluramine shows clearly that these doses of the drugs exert markedly different effects on food consumption. Amphetamine has a short time course of effectiveness with the anorexic action virtually complete by 1·5 h after which time the food intake accelerates and begins to catch up with the controls. On the other hand, fenfluramine is much longer acting and the suppressive effect on feeding is maintained for several hours after injection. These profiles illustrate how discrete food tests sampling different portions of the feeding curves would lead to ambiguous conclusions about the relative anorexic potencies of the drugs. For example, a 1 h feeding test would have shown that amphetamine was more potent than fenfluramine ($t = 4{\cdot}42$, $df = 2$, $P < 0{\cdot}05$), whereas a 2 h test would have indicated that the drugs had equivalent potency ($t = 0{\cdot}93$, $df = 2$, $P > 0{\cdot}3$), and a 3–4 h test would have suggested that, for these particular doses, fenfluramine was slightly more effective than amphetamine ($t = 3{\cdot}21$, $df = 2$, $P < 0{\cdot}1$).

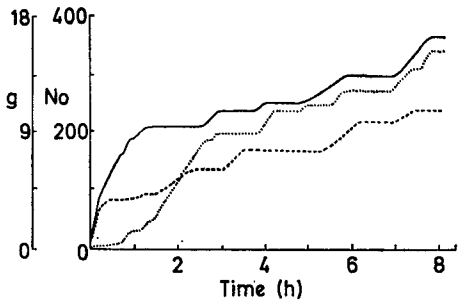

FIG. 1. Continuous records of the time course of food intake following injections of · · · · · amphetamine (1·75 mg kg^{-1}) – – – – –, fenfluramine (3·75 mg kg^{-1}) and —— control saline solution. In previous discrete food tests these doses had been shown to produce a 50% reduction in food intake measured over a 2 h feeding period. Ordinates—cumulative food intake (g) and number of pellets (No).

The feeding profiles also suggest that the drugs have different modes of action, for examination of the early portion of the graph in Fig. 1 shows that amphetamine produces a powerful initial suppression of eating followed by the release of this suppression and the resumption of normal feeding. At this time the slope of the amphetamine curve indicates that the rate of food consumption is similar to that displayed initially after control injection. It seems therefore that amphetamine acts to postpone the onset of feeding. In contrast, fenfluramine appears to allow eating to begin and to proceed normally and then acts to terminate prematurely the initial bout of feeding.

These observations were confirmed by the results of the second phase of the experiment. Fig. 2 shows the feeding profiles for three doses of amphetamine

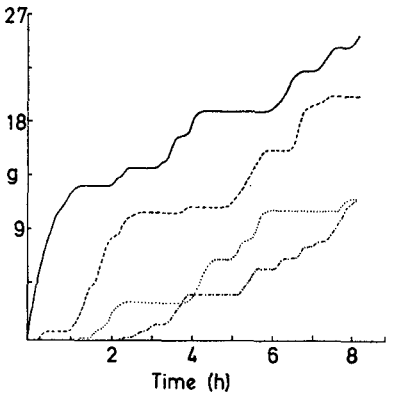

FIG. 2. Anorexic profiles for 3 doses of amphetamine. The curves are constructed from the pooled data of 3 animals. —— control, - - - - 1·0, · · · · 2·5 and -·-·- 5·0 mg kg^{-1} amphetamine. Ordinate—cumulative food intake (g).

and it is apparent that the major effect of amphetamine is to delay the onset of eating with the latency between drug injection and initiation of feeding being proportional to the dose of drug administered. For fenfluramine, the characteristic profile observed above is again in evidence at all dose levels (Fig. 3). For weak and strong doses of fenfluramine feeding is initiated normally and then curtailed with the duration of the initial bout of eating being approximately proportional to the dose injected.

The profile of amphetamine is consistent with the description of this drug as an appetite inhibitor and is in keeping with general expectations about the action of anorexic drugs. However, the effect brought about by fenfluramine was not anticipated nor was the preservation of an initial fast rate of food

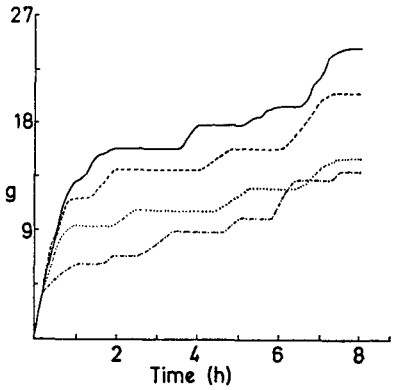

FIG. 3. Temporal profile of the inhibition of food intake following fenfluramine administration. Unlike amphetamine, this drug does not delay the onset of eating. It is noticeable that the size and duration of the initial bout of eating is related to the dose of fenfluramine injected. —— Control, - - - - 1·0, · · · · 2·5 and -·-·- 5·0 mg kg^{-1} fenfluramine. Ordinate—cumulative food intake (g).

consumption in accord with conceptions of anorexic capacity. It seemed possible that this delayed effect of fenfluramine was simply due to the rate of tissue uptake of fenfluramine or to the rate of conversion of fenfluramine to the metabolite norfenfluramine which also inhibits food intake (Beregi, Hugon & others, 1970). In previous studies which compared blood concentrations of fenfluramine and norfenfluramine with feeding activity, we have shown that the prolonged action of fenfluramine in rats is due in part to rising norfenfluramine concentrations (Blundell & others, 1975) but that norfenfluramine alone cannot account for the earlier inhibitory effect of fenfluramine (Blundell & Campbell, 1975). However, it appeared possible that the termination of the initial bout of eating following fenfluramine injection was due to the combination of rising concentrations of fenfluramine and norfenfluramine. This possibility was investigated in the second experiment.

MATERIALS AND METHODS (Exp. II)

Subjects and apparatus were as in the first experiment.

Design and procedure

If the inhibitory action of fenfluramine on food intake is delayed until critical blood or brain concentration of the drug (together with its metabolite) have been achieved, then it follows that the onset of this inhibition should be brought forward by lengthening the interval between drug injection and access to food. In Fig. 3 it can be seen that the

2·5 mg kg^{-1} dose of fenfluramine creates a plateau of inhibition in the feeding profile after about 1 h. Therefore if access to food is delayed for 1 h after injection then this plateau should occur earlier in the feeding profile. Indeed this procedure might be expected to convert the fenfluramine pattern to a profile of initial feeding suppression similar to that of amphetamine. Accordingly, a 2·5 mg kg^{-1} dose of fenfluramine was injected at intervals of 30, 60 and 90 min before access to food was permitted. The order of these intervals was counter-balanced among the animals, and the deprivation conditions, the injection procedure and the monitoring period were identical to those employed in the first experiment.

RESULTS AND DISCUSSION (Exp. II)

There was no appreciable difference between the temporal feeding profiles for fenfluramine administered with differing injection latencies (largest $t = 1{\cdot}12$ $df = 2$, $P > 0{\cdot}2$). In other words, delaying access to food after fenfluramine injection does not bring forward the onset of inhibition over feeding. It seems therefore that this phenomenon is not a simple function of increasing availability of fenfluramine in plasma nor of the conversion of fenfluramine to some active metabolite. Instead, it appears that a certain amount of food may require to be consumed in order for the inhibitory action of fenfluramine to occur. This suggestion raises the possibility that fenfluramine exerts its inhibition over feeding by enhancing feedback signals from the consumption of food. Since the phenomenon whereby feeding is terminated by the consequences of food ingestion is commonly termed satiation, fenfluramine may act to promote satiety. Experiment III investigated this possibility further.

Experiment III

In the previous experiments evidence for the mode of action of amphetamine and fenfluramine has been derived from the continuous recording of food intake following injections into food-deprived animals. However, the detailed feeding information provided by the eatometer lends itself readily to the analysis of feeding patterns in animals with free access to food when they will satisfy their requirements by consuming about 8 to 13 separate meals over 24 h (Richter, 1927), the meals varying considerably in size with larger meals being consumed during the dark phase of a light-dark cycle (Le Magnen & Tallon, 1966). It follows that any treatment which hastens the onset of satiety should lead to the premature termination of meals and consequently give rise to a general decline in meal size over the period for which the treatment is active. Accordingly, the present experiment examined the effect of amphetamine and fenfluramine on meal taking patterns in non-deprived rats.

METHODS (Exp. III)

Design and procedure

For the duration of this experiment three male hooded rats, 358–381 g, experimentally naive, lived permanently in the experimental chambers and received all their food via eatometers. At no time was food withheld. For three weeks before the administration of drugs the daily meal profiles were observed to ensure consistent feeding patterns had become established, and sham injections were periodically given. For the experiment proper, three injections were given [(+)-amphetamine, 5·0 mg kg^{-1}; (±)-fenfluramine, 10·0 mg kg^{-1}; and 0·9% w/v NaCl] and each animal received each condition twice. A counterbalanced order of drug presentation was used, and for this part of the study the drugs were administered orally 2 h before the beginning of the dark phase of the light–dark cycle. The meal patterns were monitored for 24 h from the time of drug administration. At least 72 h intervened between successive dosings to allow any disturbance of the natural meal taking profile to disappear.

For each animal the 24 h feeding records were analysed and the number of meals, the sizes of individual meals and the duration of inter-meal intervals were calculated. Since Kissileff (1970) has shown that for normal rats with free access to food, meal distribution is independent of the criteria used to separate meals from each other, a 15 min criterion was arbitrarily chosen for the present analysis. That is, a meal was defined as any distinct bout of eating separated from other eatometer readings by an interval of at least 15 min. From the data the distributions of meal sizes and inter-meal intervals were plotted for individual animals under each condition and the median values of each parameter were calculated. The median statistic was used in preference to the mean to avoid any distortion of the average due to the known non-normal distributions of meal sizes and inter-meal intervals (e.g. Kissileff, 1970). Differences between conditions were compared using the Friedman non-parametric analysis of variance.

RESULTS AND DISCUSSION (Exp. III)

The 15 min criteria for separating bouts of eating resulted in meals being clearly defined and during the course of the experiment the rats consumed between 8 and 12 meals in any 24 h. Fig. 4 shows the distribution of inter-meal intervals measured under each of the three conditions and although amphetamine and fenfluramine brought about some modification of the curve when compared with the control, these departures were not statistically significant. The median inter-meal intervals were 105, 90 and 135 min for control, fenfluramine and amphetamine conditions respectively ($\chi^2 = \cdot 17$, NS), and all the medians fell within the same portion of the curve (between 5 and 10 15-min periods).

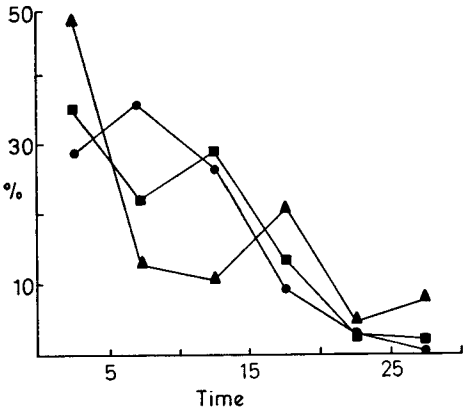

FIG. 4. Distribution of inter-meal intervals measured during 24 h periods following amphetamine, fenfluramine or saline injections. Each curve is based on data from 144 h of continuous recording. ●—● Control, ■—■ amphetamine, ▲—▲ fenfluramine. Ordinate—percent of total intervals. Abcissa—duration of inter-meal interval (each unit represents one 15 min period).

The distribution of meal sizes is shown in Fig. 5 and a clear dissociation between the effects of the two drugs is apparent. Whereas amphetamine produced little effect on this measure, fenfluramine markedly influenced the distribution of meal sizes and overall substantially reduced the sizes of meals consumed*. The median meal sizes were 2·6, 2·4 and 1·6 g for saline, amphetamine and fenfluramine conditions respectively. Although the probability value of the χ^2 statistic for comparing these scores is given as 0·19 it is clear that with a small N, achievement of the customary 5% level of significance

* This effect of fenfluramine on meal size has also been observed in an independent investigation—S. Jaggers, personal communication.

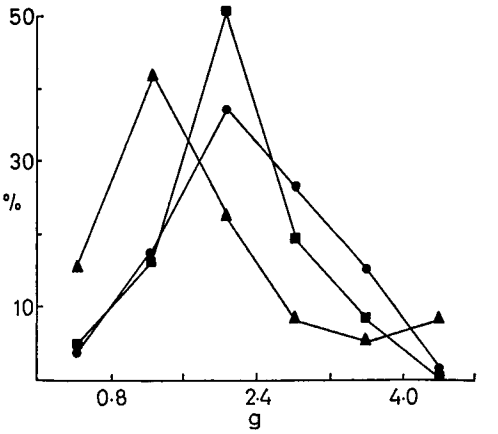

FIG. 5. Distribution of meal sizes measured during 24 h periods following saline, amphetamine or fenfluramine injections. This meal pattern analysis is based on 144 h of continuous recording of food intake and the median meal sizes were 2·6, 2·4 and 1·6g for the three drug treatments respectively. See text for details. ●—● Control, ■—■ amphetamine, ▲—▲ fenfluramine. Ordinate—percent of total meals. Abcissa—meal size (g).

can only arise when all subjects exhibit the same rank order for each of the three conditions. (See Siegel, 1956). For the purpose of this analysis it is important to recognize that fenfluramine brought about a small but definite diminution in meal size in *each* of the subjects.

It is worth drawing attention to one further methodological aspect arising from this experiment which may influence the interpretation of data collected under these conditions. In assessing the influence of pharmacological manipulation upon meal patterns, it is important to keep in mind the possibility of combining data from a period of intense drug activity with data from a subsequent period when animals are recovering from the drug effect and may be expected to increase consumption to make up for previously reduced intake. Although the results obtained in this experiment with amphetamine must clearly be subjected to this consideration, it is important to point out that the dose of fenfluramine was specifically chosen to ensure that effective blood concentrations of the drug or an active metabolite (Blundell & others, 1975) were present during the entire collection period.

GENERAL DISCUSSION

The use of a device for the continuous monitoring of food intake has been found useful for providing detailed information on the action of anorexic drugs. Such a technique seems preferable to the use of

discrete time periods for sampling food intake which may fail to reveal critical features of drug action. In particular, experiment I demonstrated that doses of amphetamine and fenfluramine which had been shown to exert comparable suppressive effects on food intake measured during 2 h, actually display quite distinctive anorexic profiles which become apparent when feeding is monitored continuously. This dissociation between the drugs seems to represent differences in their *mode* of action. The term mode is here used to signify identifiable characteristics in the observed manner in which the drugs inhibit feeding, and the term itself need not embody the suggestion that different aspects of the feeding process are associated with different modes, or that differing actions of the drugs on neurochemical feeding systems constitute the mechanisms which give rise to particular modes. However, in view of the marked contrast between the actions of amphetamine and fenfluramine on brain chemistry (Garattini, Buczko & others, 1975) and following brain lesions (Blundell & Leshem, 1975a), it is worth considering the implications of the differences between their modes of action observed above.

In deprived rats (exp. I) the main effect of amphetamine was to delay the initiation of food consumption. Since most definitions of hunger incorporate, among other ideas, the tendency to begin eating, this effect of amphetamine may be characterized as an effect upon hunger. In addition. although amphetamine produced no striking effect on meal patterns of satiated animals (exp. III), it is noticeable that this drug condition was associated with the largest median inter-meal interval. This observation, which indicates some delay in meal initiation brought about by the drug, is quite in keeping with an action to postpone the onset of eating. It is possible that this feature could be wholly accounted for by the rapid rise of amphetamine to a peak concentration in the blood (Blundell & others, 1975) and by the rapid inactivation to *p*-hydroxyamphetamine which is believed to have little central activity (Goodman & Gilman, 1970). However, a proposed suppressive action of amphetamine on hunger is quite consistent with the reported effects of amphetamine on neural mechanisms involved in food motivational behaviour. Injections of the drug directly into the brain have shown that food intake is depressed by the action of amphetamine at the lateral hypothalamus (e.g. Leibowitz, 1970; Blundell & Leshem, 1973; Leibowitz, 1975), while lesions of the lateral hypothalamus can abolish the anorexic effect of amphetamine (Carlisle, 1964; Blundell & Leshem, 1974a). Since the lateral hypothalamus is a zone believed to be crucially implicated in the manifestation of many features of hunger (see Wise, 1974, for discussion of this issue), a wide range of evidence lends support to the idea that the observed effect of amphetamine on feeding profiles may be identified as an effect upon hunger which could be brought about by an action upon central hunger mechanisms.

A comparison of the observed anorexic profiles in experiment I showed that the mode of action of fenfluramine stood in marked contrast to that of amphetamine. Whereas amphetamine suppressed the onset of eating, under the influence of fenfluramine feeding commenced normally but the drug hastened the termination of the initial bout of eating. The patterns of inhibition of eating brought about by these two drugs therefore appear to be the opposite of each other. In turn, these contrasting patterns may be associated with actions of the drugs upon differing components of the feeding process, amphetamine being characterized by a suppression of hunger while fenfluramine facilitates satiety. In this context satiety is being used to signify more than the simple reduction in weight of food consumed for the term correctly should be applied to the cessation of food consumption brought about by consequences of the act of eating itself. In studies where anorexic activity is assessed by measuring the amount of food consumed in a given period, it is impossible to reveal whether a reduction in food consumption has been brought about by an action on hunger or satiety.

The observed effect of fenfluramine on the pattern of meal taking in freely feeding animals (exp. III) supported the results from the earlier experiments using deprived rats. The measured reduction in the overall size of meals by fenfluramine is quite consistent with an effect of the drug intensifying processes bringing about the cessation of feeding, and the possibility arises that fenfluramine begins to exert its main inhibitory action over feeding only when eating has been initiated. In addition, it seems that this effect may be brought about by the action of fenfluramine on brain chemistry. Many reports have suggested that alterations in brain 5-hydroxytryptamine (5-HT) are associated with the effect of fenfluramine on food intake in animals (see Reuter, 1975) and also in man (Shoulson & Chase, 1975). Morevoer, there is now an accumulation of evidence indicating the existence of a brain 5-HT mechanism inhibitory for feeding (see Blundell & Leshem, 1975b for references). Significantly, whereas 5-HT agonists lead to the inhibition of feeding, 5-HT antagonists have been shown to increase food intake by dissipating the

effects of satiety (Blundell & Leshem, 1974b). Fenfluramine, which may have an indirect (Garattini & others, 1975) or a direct (Funderburk, Hazelwood & others, 1971; Sugrue, Goodlet & McIndewar, 1975) effect on 5-HT systems, brings about a decrease in meal size (exp. III above), while 5,6-dihydroxytryptamine, which produces a degeneration of central 5-HT terminals, may lead to an increase in meal size (Diaz, Ellison & Masuoka, 1974). Accordingly, serious consideration must be given to the hypothesis that fenfluramine exerts an inhibitory action on food intake by acting upon a 5-HT satiety mechanism. The 5-HT system could act by mediating in the feedback of signals contingent upon food consumption, and the existence of such a system is in keeping with models of the feeding process incorporating specific roles for dopamine, noradrenaline and 5-HT (Blundell, 1975b). However, this hypothesis will have to be revised if it is subsequently shown that the action of fenfluramine is partially or wholly mediated by non-5-HT systems.

The results of this study may have practical significance for they clearly show that, with respect to behavioural action, anorexic drugs constitute a heterogenous group of chemicals. It seems likely that future research will identify further drugs which reduce food intake by suppressing the onset of eating (the amphetamine pattern) whereas others may facilitate satiety (fenfluramine pattern). These findings may have important implications for the treatment of obesity for clinical evidence indicates that the obese population represents a heterogenous group of individuals. Some observers have noted that 'a characteristic complaint is an inability to stop eating once they have started' (Stunkard, 1968), whereas others report that 'obese individuals display behaviour characteristic of hunger' (Nisbett, 1972). Consequently, the possibility arises of administering hunger-suppressant drugs to obese people who show enhanced hunger, and satiety facilitating drugs to individuals who display defective satiety.

REFERENCES

BEREGI, L. G., HUGON, P., LE DOUAREC, J. C., LAUBIE, M. & DUHAULT, J. (1970). In: *Amphetamines and related compounds*. New York: Raven Press.

BLUNDELL, J. E. (1975a). *Nutrition (Lond.)*, **29**, 5–18.

BLUNDELL, J. E. (1975b). *Physiological Psychology*, p. 79. London: Methuen.

BLUNDELL, J. E. & CAMPBELL, D. B. (1975). *Br. J. Pharmac.*, **55**, 261P.

BLUNDELL, J. E., CAMPBELL, D. B., LESHEM, M. B. & TOZER, R. (1975). *J. Pharm. Pharmac.*, **27**, 187–192.

BLUNDELL, J. E. & LESHEM, M. B. (1973). *Br. J. Pharmac.*, **47**, 183–185.

BLUNDELL, J. E. & LESHEM, M. B. (1974a). *Eur. J. Pharmac.*, **28**, 81–88.

BLUNDELL, J. E. & LESHEM, M. B. (1974b). *Proc. Vth Int. Cong. Physiol., Food and Fluid Intake*, p. 37.

BLUNDELL, J. E. & LESHEM, M. B. (1975a). *Postgrad. med. J., Suppl.* 1, **51**, 45–54.

BLUNDELL, J. E. & LESHEM, M. B. (1975b). *J. Pharm. Pharmac.*, **27**, 31–37.

CARLISLE, J. (1964). *J. Comp. Physiol. Psychol.*, **58**, 47–54.

COX, R. H. & MAICKEL, R. P. (1972). *J. Pharmac. exp. Ther.*, **181**, 1–9.

DIAZ, J., ELLISON, G. & MASUOKA, D. (1974). *Psychopharmacologia*, **37**, 67–79.

FUNDERBURK, W. H., HAZELWOOD, J. C., RUCKHART, J. T. & WARD, J. W. (1971). *J. Pharm. Pharmac.*, **23**, 468–470.

GARATTINI, S. (1972). *La Vie medicale au Canada francais*, 15, January 21–27.

GARATTINI, S., BUCZKO, W., JORI, A. & SAMANIN, R. (1975). *Postgrad. med. J., Suppl.* 1, **51**, 27–35.

GOODMAN, L. S. & GILMAN, A. (1970). In: *The Pharmacological basis of Therapeutics*, New York: Macmillan.

KISSILEFF, H. R. (1970). *Physiol. Behav.*, **5**, 163–173.

LEIBOWITZ, S. F. (1970). *Proc. Nat. Acad. Sci.*, **67**, 1063–1067.

LEIBOWITZ, S. F. (1975). *Brain Res.*, **84**, 160–7.

LEMAGNEN, J. & TALLON, S. (1966). *J. Physiol. (Paris)*, **58**, 323–349.

NISBETT, R. E. (1972). *Psychol. Rev.*, **79**, 433–453.

REUTER, C. J. (1975). *Postgrad. med. J., Suppl.* 1, **51**, 18–27.

RICHTER, C. (1927). *Quart. Rev. Biol.*, **2**, 307–343.

SEIGEL, S. (1956). *Non-parametric statistics for the behavioural sciences*, New York: McGraw-Hill.

SHOULSON, I. & CHASE, T. N. (1975). *Clin. Pharmac. Ther.*, **17**, 616–621.

STUNKARD, A. J. (1968). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **27**, 1367–1373.

SUGRUE, M. F., GOODLET, I. & MCINDEWAR, I (1975). *J. Pharm. Pharmac.*, **27**, 950–953.

WISE, R. A. (1974). *Brain Res.*, **67**, 187–209.