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Comparison of the time course of the anorectic effect of fenfluramine and amphetamine with drug levels in blood

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Time courses of the suppressive effects on food intake of (+)-amphetamine and (±)-fenfluramine in deprived rats were found to be different. Amphetamine displayed a potent initial action which rapidly decayed, and this behavioural effect was consistent with the measured blood concentration of amphetamine which showed a peak at 1 h followed by rapid clearance. For fenfluramine, the initial suppression of eating was maintained over several hours and was, for the first hour, related to the blood concentration of fenfluramine but later to an active metabolite, norfenfluramine. The study shows how drug-induced changes in feeding behaviour fluctuate over time and illustrate how single measures of food intake may overlook information about the effectiveness of anorectic drugs.

In many studies on the capacity of drugs to depress feeding, the magnitude of the drug-induced anorexia has been derived from the depression of food intake observed during a brief feeding period (usually 2 h). Few experiments have measured the time course as well as the magnitude of anorectic action and conflicting results have been produced when the effects of amphetamine and fenfluramine have been examined over varying periods of time (see for example Le Douarec, Schmitt & Laubie, 1966; Bizzi, Bonaccorsi & others, 1970). The results of studies on the interaction of anorectic drugs with neurotransmitter modulators may depend upon the length of the feeding period (Blundell, Latham & Leshem, 1973), it therefore seems necessary to investigate whether the intervals most frequently used for the measurement of food intake are appropriate to the duration of action of anorectic drugs. Moreover, as the behavioural action of drugs may be related to the amount of drug in the body, it is important to examine whether or not changes in behavioural effectiveness may be explained by fluctuations in blood concentrations of drug. We have therefore compared the time course of the anorectic activity of amphetamine and fenfluramine with the time course of their blood concentrations. However, the specification of relations between behavioural changes and injected drugs is often complicated by the conversion of the drug to behaviourally active metabolites. This is so with fenfluramine which is metabolized to norfenfluramine, a compound with marked anorectic properties (Beregi, Hugon & others, 1970). For this reason, temporal changes in the blood concentration of norfenfluramine were monitored in addition to those of amphetamine and fenfluramine.

METHODS

Food intake measurement

Animals. Male black hooded rats, about 350 g, were housed in single cages.

Procedure and design. During the course of the experiment, animals were given feeding tests after a period of 16 h food deprivation. Each deprivation period was followed by a full day in which animals were allowed continual access to food and during which measurements of consumption (to the nearest 0·1 g) were taken after 1, 4, 8 and 24 h. Food was presented in small trays in pellet form and spillage was collected on tissue paper placed below the wire-grid floors. For at least one month before drug injections, animals were rendered docile by being handled daily. In addition, the food deprivation schedule was begun 10 days before the start of drug injections to allow time for animals to become accustomed to the regime, 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. During the injection series, 7 drug treatments were administered: 1·0, 2·5 and 5·0 mg kg^{-1} (+)-amphetamine sulphate; 2·5, 5·0 and 10·0 mg kg^{-1} (±)-fenfluramine hydrochloride and 0·9% w/v NaCl. A within-subjects design was used and animals received the 7 drug conditions in counterbalanced order with at least 72 h intervening between successive injections. The results were analysed with a one-way analysis of variance procedure for repeated measures and, where necessary, the individual means were compared by Newman-Keuls technique, (Kirk, 1970). Pooled comparisons between blood conditions were made by the Student's *t*-test.

Measurement of drug concentrations in blood

Animals and procedures. Male hooded rats, housed in single cages, had body weights similar to those animals in the food intake study. Since it seemed possible that changes in food deprivation state could influence the rate of absorption of drugs, blood was taken from these animals under the identical deprivation conditions (16 h) used during the measurement of food intake. After the administration of 10 mg kg^{-1} doses of the drugs, blood samples were collected at intervals of 1, 4, 8 and 24 h after injection and at each period, animals were briefly anaesthetized with carbon dioxide and approximately 0·5 ml of blood was taken by cardiac puncture.

Analysis. The blood was made alkaline with 0·5 ml of 5N sodium hydroxide and extracted by shaking mechanically for 5 min with an equal quantity of ether and 100 μl of internal standard solution (0·5 mg % *NN*-diethylanaline in chloroform). After centrifugation at 4000 rev min^{-1}, the ether phase was transferred to a finely tapered centrifuge tube containing 0·3 ml of N sulphuric acid. The tube and contents were shaken for 2 min and after centrifugation, the ether layer was removed. Approximately 0·3 ml of clean ether was added and the aqueous phase basified with 0·2 ml of 5N sodium hydroxide and the drugs were extracted into the ether by whirlimixing for one min. After brief centrifugation, the ether layer was transferred into a clean finely tapered centrifuge tube, avoiding transference of water, and evaporated to approximately 50 μl under nitrogen at 20°; 3–4 μl was injected on to the gas liquid chromatograph. The evaporation of the ether must be carefully controlled since evaporation to dryness causes preferential volatilization of the internal standard which renders subsequent results incorrect (Campbell, 1970).

Calibration curves were constructed by running known standards of fenfluramine, norfenfluramine and amphetamine through the procedure. The reproducibility of the method was measured by performing five duplicate assays on samples containing

150 ng ml^{-1} of amphetamine, fenfluramine and norfenfluramine. For all compounds, the standard error of the mean figure was less than 4%.

G.l.c. conditions. The gas liquid chromatograph used was a Pye 104, equipped with dual flame ionization detectors. The glass columns (5 m × 4 mm i.d.) were packed with 10% Apiezon L and 10% potassium hydroxide on AW/DCMS Chromasorb W. The column oven temperature was at 150° with an injection port temperature at 210°. The gas flow rates were; N_2:70 ml min^{-1}, H_2:35 ml min^{-1}, O_2:320 ml min^{-1}. Under these conditions, the relevant retention times of the drugs were norfenfluramine, 2·4 min, amphetamine, 3·4 min and fenfluramine 4·1 min.

RESULTS

Food intake

Analysis of variance showed that the anorectic drugs produced a significant effect on food intake at all feeding periods (smallest $F = 3{\cdot}7$; d.f. = 6 and 66; $P < 0{\cdot}01$). The results in Table 1 make possible an assessment of both the initial magnitude of the anorexia produced by the drugs and the rate at which their anorectic effects decline. All doses of amphetamine and fenfluramine gave rise to the largest reduction in food intake during 0–1 h and the magnitude of the effect was proportional to the dose of drug. However, the anorectic effect of amphetamine in doses of 1·0 and 2·5 mg kg^{-1} i.p. waned by the second feeding period (1–4 h) the animals eating marginally more than saline-injected animals (118% and 125% for the two doses respectively). At the end of the 4–8 h period rats given 5·0 mg kg^{-1} of amphetamine had begun to eat more than saline controls. Thus, amphetamine produces a powerful initial anorectic effect which rapidly decays. The pattern of anorectic activity of fenfluramine is different. With fenfluramine, the initial magnitude of the anorectic effect is less than for amphetamine but the effect is maintained longer and it is not until the 8–24 h period that treated rats begin to eat more than saline controls.

These findings show how the length of the feeding period may influence the apparent anorectic activity of a drug: when the feeding period is short (0–1 h) amphetamine could be shown to exert a markedly greater appetite depressant effect than fenfluramine ($t = 4{\cdot}3$; d.f. = 11; $P<0{\cdot}01$) but for an extended feeding period (0–8 h), the opposite could be demonstrated ($t = 3{\cdot}9$; d.f. = 11; $P < 0{\cdot}01$).

Blood levels of drugs

Analysis of the amount of drug in blood at 4 times after injection showed clear differences in the pharmacokinetics of amphetamine and fenfluramine. The measured half-life of amphetamine was 1·5 h and Fig. 1 shows that this drug reaches a peak concentration in blood at 1 h followed by a rapid clearance so that little remains after 8 h. The Figure also shows the change in anorectic potency occurring over time following drug administration. It can be seen that the temporal changes in anorectic potency are closely related to those in the blood concentration with maximal anorectic potency coinciding with the maximal amount of amphetamine in blood, and with the rebound eating effect being apparent at 8 h when the blood concentration has fallen almost to zero.

Fig. 2 shows the blood concentrations of fenfluramine and norfenfluramine after administration of fenfluramine. The measured half-life of fenfluramine was 2·6 h and although the peak concentration was observed at the 1 h sampling period, the

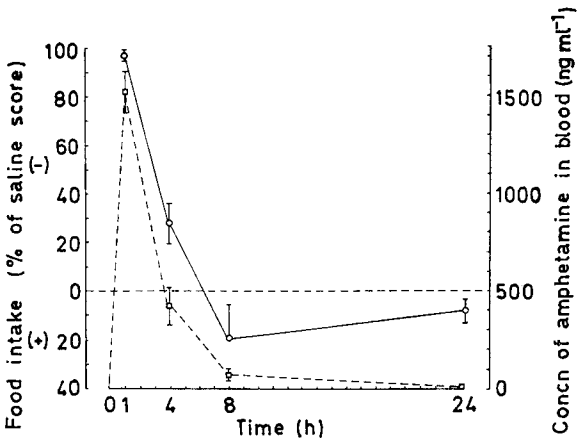

FIG. 1. Comparison of the food intake as a percentage of saline controls ○——○, n = 12, ((+)-amphetamine sulphate 5 mg kg^{-1}, i.p.) and blood concentrations □- - -□, n = 6, [(+)-amphetamine sulphate 10 mg kg^{-1}, i.p.] in 16 h deprived rats.

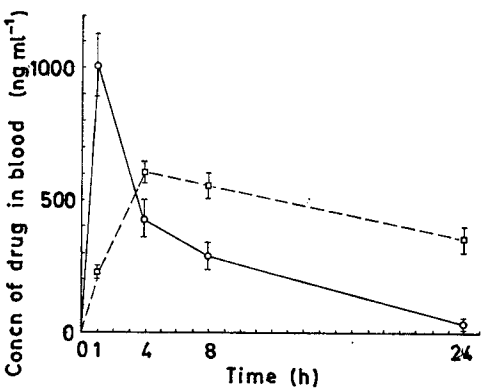

FIG. 2. Blood concns of fenfluramine ○——○ and norfenfluramine □- - -□ following administration of (±)-fenfluramine 10 mg kg^{-1} intraperitoneally.

amount in the blood declines at a slower rate than does amphetamine. However, the blood concentrations of norfenfluramine show a different pattern, with the proportion of norfenfluramine to fenfluramine gradually increasing over time. The slow decline in the amount of norfenfluramine in blood is consistent with its measured half-life (14·0 h) in rats (Campbell, Blundell, Lesham and Tozer,—unpublished data) and since it itself exhibits anorectic properties, the conversion of fenfluramine to norfenfluramine provides an explanation for the prolonged anorectic action of fenfluramine compared with amphetamine (see Table 1). This belief is supported by the finding that the closest relation between anorectic potency and the amount of drugs in the blood is given when the concentrations of fenfluramine and norfenfluramine are added together (Fig. 3). Thus, the anorectic effect of fenfluramine in rats seems to be mediated by both fenfluramine and norfenfluramine, with fenfluramine responsible for the initial anorectic effect and norfenfluramine responsible for the prolonged anorectic action.

Table 1. *Food intake (g) following intraperitoneal injections given 30 min. before the start of the food tests.* Values given are means (n = 12 in each case) and the figures in parentheses show the drug scores expressed as a percentage of the saline scores.

Treatment	Dose	Periods of food consumption (h)			
	mg kg^{-1}	0–1	1–4	4–8	8–24
Saline		7·8	7·2	7·9	21·1
Amphetamine	1·0	4·1 (56)	8·5 (118)	9·2 (116)	21·0 (99)
	2·5	0·4 (5)	9·0 (125)	8·5 (107)	22·4 (106)
	5·0	0·3 (4)	5·0 (69)	9·3 (118)	22·6 (106)
Fenfluramine	2·5	5·6 (71)	6·3 (88)	7·0 (88)	23·3 (109)
	5·0	4·1 (52)	5·2 (73)	5·7 (72)	24·0 (113)
	10·0	2·6 (33)	2·3 (31)	4·2 (53)	27·0 (127)

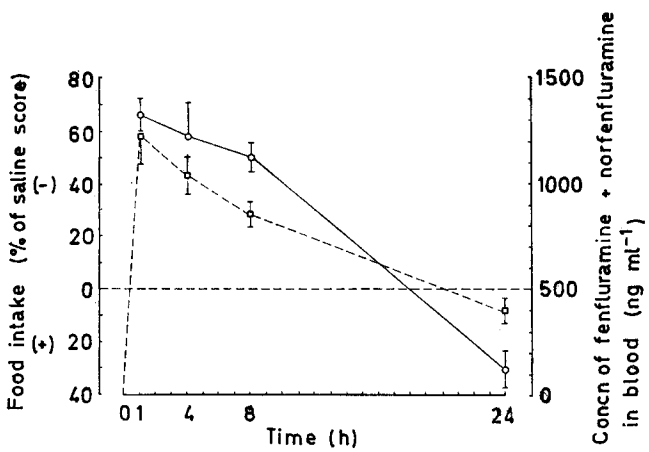

FIG. 3. Comparison of the food intake as a percentage of saline controls ○——○ (n = 12) and the summed blood concns of fenfluramine and norfenfluramine □– – –□ (n = 6) following intraperitoneal injection of (±)-fenfluramine hydrochloride (10 mg kg^{-1}).

DISCUSSION

The results of this study show clearly that the pattern of anorectic activity of amphetamine and fenfluramine is different in initial magnitude and time course. These differences have implications for deriving doses which are functionally equivalent. Reference to Table 1 shows that for the 0–1 h feeding period, a 52% food intake is achieved by amphetamine 1·0 mg kg^{-1} and fenfluramine 5·0 mg kg^{-1}. However, over 0–4 h, it can be calculated that amphetamine 2·5 mg kg^{-1} now produces an equivalent food intake (62%) with fenfluramine 5·0 mg kg^{-1}, over 0–8 h, it is the 5·0 mg kg^{-1} dose of amphetamine that gives rise to an anorectic effect equivalent to 5·0 mg kg^{-1} of fenfluramine.

Thus, the derivation of doses of drugs which are considered functionally equivalent in anorectic potency depends crucially upon the length of the feeding period. In fact, the data in Table 1 indicate how an arbitrary choice of time intervals could demonstrate that amphetamine was more potent than fenfluramine, less potent, or failed to differ in potency. In addition, since the behavioural effects of drugs alter markedly with time, planned comparisons between anorectic potency and the strength

of other behavioural effects (e.g. Cox & Maickel, 1972) should ideally be carried out over similar time intervals.

The difference between the behavioural profiles of amphetamine and fenfluramine is consistent with the observed time courses of changes in the blood concentration of the drugs and their metabolites. The effect of amphetamine seems to be characterized by a high initial hunger suppressive action with rapid decline in anorectic potency; these features may be accounted for by its short half-life (1·5 h) and by its inactivation in the rat to *p*-hydroxyamphetamine which is thought to have little central activity (Goodman & Gilman, 1970).

On the other hand, the prolonged anorectic effect of fenfluramine seems to be due to the anorectic potency of fenfluramine itself together with the suppressive effect on food intake of norfenfluramine. However norfenfluramine cannot account for the full anorectic effect of fenfluramine (Campbell, Blundell & others, unpublished. Moreover, Campbell (1973) has shown that in man, the conversion of fenfluramine to norfenfluramine is of less importance and consequently the relative blood concentrations of norfenfluramine are markedly lower in man than in rat. Certainly in man, it would appear that fenfluramine alone is responsible for the anorectic (Silverstone, Fincham & Campbell, 1974) and clinical effects of the drug (Hossain & Campbell, 1974).

Our results point to difficulties which may arise in studies on mechanism of action which use but a single feeding period. For, while a brief measuring interval may be appropriate for investigations of amphetamine where the anorectic activity (in response to moderate doses) is limited to a short period after injection, the same interval fails to encompass the pattern of anorectic activity of fenfluramine. Consequently, studies on anorectic action in drugs like fenfluramine may neglect information by focussing on short discrete epochs of eating behaviour.

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REFERENCES

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

BIZZI, A., BONACCORSI, A., JESPERSEN, S., JORI, A. & GARATTINI, S. (1970). In: *Amphetamines and related compounds.* New York: Raven Press.

BLUNDELL, J. E., LATHAM, C. J. & LESHAM, M. B. (1973). *J. Pharm. Pharmac.*, **25**, 492–494.

CAMPBELL, D. B. (1970). *J. Chromatog.*, **49**, 442–447.

CAMPBELL, D. B. (1973). *La Vie medicale au Canada francais*, **15**, January 19–21.

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

LE DOUAREC, J. C., SCHMITT, H. & LAUBIE, M. (1966). *Archs int. Pharmacodyn. Thér.*, **161**, 206–232.

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

HOSSAIN, M. & CAMPBELL, D. B. (1974). *Postgrad. med. J.*, in the press.

KIRK, R. E. (1970). *Experimental Design—procedures for the behavioural sciences.* Prentice Hall: London.

SILVERSTONE, T. J., FINCHAM, J. & CAMPBELL, D. B. (1974). *Postgrad. med. J.*, in the press.