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POSSIBLE RELATIONSHIPS BETWEEN CHANGES IN BODY WEIGHT SET-POINT AND STRESS METABOLISM AFTER TREATING RATS CHRONICALLY WITH D-FENFLURAMINE

EFFECTS OF FEEDING RATS ACUTELY WITH FRUCTOSE ON THE METABOLISM OF CORTICOSTERONE, GLUCOSE, FATTY ACIDS, GLYCEROL AND TRIACYLGLYCEROL

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Abstract—Rats were maintained on a corn oil diet and treated with D-fenfluramine at doses of 2.5 mg/kg twice a day for 11 days or with 10 mg or 25 mg/kg once a day for 12 days. The lower dose of D-fenfluramine produced no marked changes in body weight and after 11 days of treatment the weights of the rats on average were only 2% lower than the controls. The food intake of these rats was only decreased on the first day. The two higher doses of D-fenfluramine decreased the food consumption for about 3 days but thereafter it was similar to that of the control rats. The body weight of these rats fell on the first day, but after about four days the gain in body weight paralleled rather than approached that of the control rats. Increasing the dose of D-fenfluramine progressively decreased the relative size of the epididymal fat pad. At the end of the treatment period the rats were fed acutely with fructose to increase the circulating concentrations of corticosterone and to stimulate triacylglycerol synthesis. All three doses of D-fenfluramine decreased the concentration of circulating triacylglycerol after fructose feeding. The 10 mg/kg dose also decreased the basal concentration of triacylglycerol. The two higher doses of fenfluramine decreased the rises in the circulating concentrations of corticosterone, glycerol and fatty acids that are produced by fructose feeding. The basal concentrations of these compounds in the absence of fructose feeding were not significantly affected by the 10 mg/kg dose of D-fenfluramine. The possible relationship between the effect of chronic treatment with D-fenfluramine in decreasing a metabolic stress response and lipolysis is discussed relative to its hypotriglyceridaemic action and its effect on body weight-set point. The results demonstrate that D-fenfluramine produced persistent changes in metabolism at a time when the treated rats were growing at the same rate as the control rats and when they were eating similar quantities of food.

Fenfluramine was originally introduced as an anorectic agent, and it has been widely used to treat obesity. However, it has become evident that this and related drugs have important metabolic effects which may be independent of an action in decreasing food intake. These include hypoglycaemic [1] and hypolipidaemic effects [2] and an ability to improve insulin sensitivity [2].

It has been proposed that these actions might be brought about by a decrease in the release or action of "stress" hormones, particularly glucocorticoids [2]. These latter hormones antagonize the effects of insulin on glycolysis, protein synthesis and the uptake of glucose by some tissues. They promote the breakdown of protein, and amino acids and they stimulate gluconeogenesis. However, in terms of energy deposition they facilitate the action of insulin in stimulating the synthesis of glycogen and fatty acids [3] in the liver, and the activity of lipoprotein lipase in adipose tissue [4]. Glucocorticoids also stimulate the synthesis of triacylglycerols in the liver and their secretion in very low density lipoproteins [for reviews, see 5, 6]. This effect is thought to be facilitated by the action of glucocorticoids together with cyclic AMP in increasing the rate of synthesis of phosphatidate phosphohydrolase [6, 7]. This enzyme is then activated and translocated to the membranes on which triacylglycerol is synthesized in response to an increased fatty acid availability which would arise from lipolysis in adipose tissue in a stress condition [8–10]. This mobilization of fatty acid and the increase in the phosphohydrolase activity would be prevented by a decrease in the release of stress hormones and an increase in the availability and effectiveness of insulin [6, 7].

There is some circumstantial evidence that fenfluramine may modify stress metabolism when administered chronically and that this could contribute to the antiobesity effect:

(a) Glucocorticoid-induced obesity in man is effectively treated by fenfluramine [11, 12];

(b) Fenfluramine-like drugs may be more effective in treating obesity in cases of overeating in which stress or emotional factors play a major role [13]. This latter work involved the use of tail-pinch to stimulate overeating in rats. Muscimol-induced hyperphagia, which shows some analogies to the tail pinch model is also inhibited by D-fenfluramine [14].

(c) Feeding guinea pigs with ascorbate does not affect the anorectic action of fenfluramine, but it does decrease its hypolipidaemic action [15, 16]. Ascorbate accumulates in the adrenal gland [17, 18] and it decreases the rate of steroid hormone synthesis [19]. High concentrations of glucocorticoids are found in the blood of scorbutic animals [17, 18] and this could contribute to the hypertriglyceridaemia and hypercholesterolaemia which also occurs [20];

(d) Drugs related to fenfluramine decrease the ethanol-induced increase in circulating corticosterone in rats when it is administered chronically [21, 22]. This treatment also partially prevents the effects of ethanol in increasing the activity of phosphatidate phosphohydrolase in the liver [21, 23], and in stimulating the synthesis and accumulation of hepatic triacylglycerols [21, 24];

(e) Although human obesity is not normally associated with hypercortisolism, it has been shown that post-obese women have an exaggerated cortisol output and an impaired release of growth hormone and prolactin during insulin-induced hypoglycaemia. This suggests an altered hypothalamic control [25]. It may also be significant that D-fenfluramine antagonized the hyperphagic responses in rats after administering insulin and 2-deoxy-D-glucose [26];

(f) Many of the obese animal models exhibit high concentrations of glucocorticoids and the obesity can often be partly reversed by adrenalectomy [27, 28].

The present experiments were therefore designed to investigate whether chronic administration of fenfluramine could decrease a metabolic stress response. The D-isomer of fenfluramine was selected for this work since it appears to be the more active compound in the racemate [29, 30]. It was important that the treatment with D-fenfluramine should be chronic since in the short-term fenfluramine causes the release of corticosterone in rats [31, 32] and it stimulates lipolysis in adipose tissue [1, 2]. Furthermore, it was necessary to dissociate the acute anorectic effects of the drug from the long-term effects on metabolism.

The experimental model chosen for this work was the rat fed on a high fat diet. This exaggerates stress responses compared to rats on high carbohydrate diets and often produces insulin-insensitivity [see ref. 33]. The metabolic stress was induced by feeding an acute load of fructose by stomach tube. This leads to a rapid increase in circulating corticosterone, glucose, glycerol and triacylglycerol without changing insulin concentrations [33, 34] and it is particularly pronounced when the rats are maintained on a diet rich in corn oil [33]. It has been proposed that an exaggerated corticosterone response could explain why high fat diets enhance the effects of fructose and ethanol in stimulating the secretion of very low density lipoproteins or producing a fatty liver [33]. Fructose will also provide carbon atoms for the synthesis of glucose as well as triacylglycerols.

MATERIALS AND METHODS

The sources of materials, animals and the pelleted corn oil diet have been described previously [35]. This diet provided 48% of its energy from fat, 19% from protein and 33% from carbohydrate. Its effects on the growth rates of rats, body composition, the concentrations of some metabolites in blood and brain, and the activities of some enzymes in liver, heart and adipose tissue have already been described [33, 35–37]. The methods of measurement were as described in this previous work [35] except that triacylglycerol was measured using a Triglyceride GPO-PAP Test Combination Kit as supplied by Boehringer Mannheim. The values for total glycerol were then corrected by subtracting the free glycerol as described previously [35]. D-Fenfluramine hydrochloride was supplied by Les Laboratoires Servier, 22 rue Garnier, 92200 Neuilly-sur-Seine, France.

Treatment of rats. Rats weighing 150–170 g were placed in grid-bottomed cages (four rats per cage) in a room that was maintained at about 22° and which was lit from 0800 to 2000 hr. The rats were fed on a 41B diet [35] for 7 days. The food was then changed to the pelleted corn oil diet [35]. After a further 7 days the rats were then injected intraperitoneally with the appropriate concentration of D-fenfluramine dissolved in 0.16 M sterile NaCl by using 2.5 ml of this per kg of body wt. Control rats were injected with the equivalent volume of sterile saline. The injections were performed from 0800 to 1000 hr and they were repeated each day. In the case of the rats treated with 2.5 mg of D-fenfluramine per kg, the injection was also repeated between 1600 and 1700 hr. On the morning of the final day of treatment the rats were fed by stomach tube with 1.68 M fructose at a dose of 9.5 g of fructose per kg [33, 34]. This was performed 2 hr after the last injection of D-fenfluramine. The rats were then killed at the times indicated and the blood was collected [33].

RESULTS

Effects of D-fenfluramine on weight gain and food intake. The initial part of the work was to establish appropriate doses of D-fenfluramine for the metabolic studies and to treat the rats for long enough so that food intake and body weight gain were restored. The dose of 2.5 mg of D-fenfluramine per kg twice a day produced no marked changes in body weight gain and after 11 days of treatment the weights of the treated rats were only 2% lower than the controls i.e. 5 g for a 250 g rat (Fig. 1a). This dose of D-fenfluramine only depressed food intake slightly on the first day.

A dose of 10 mg of D-fenfluramine per kg did produce a decrease in body weight that persisted for 4 days (Fig. 1a). Thereafter, body weight gain paralleled that of the control rats and it remained on average about 6% lower than the controls. Food intake was depressed for 2 days and after that it approached that for the control rats so that the intakes were not significantly different (Fig. 1b). The highest dose of D-fenfluramine (25 mg/kg) produced more dramatic changes in growth pattern and food intake compared to the 10 mg/kg dose (Fig. 1).

Effect of D-fenfluramine on the epididymal fat pad weights. The weights of the epididymal fat pads were measured when the rats were killed. These are expressed relative to the body weights of the rats

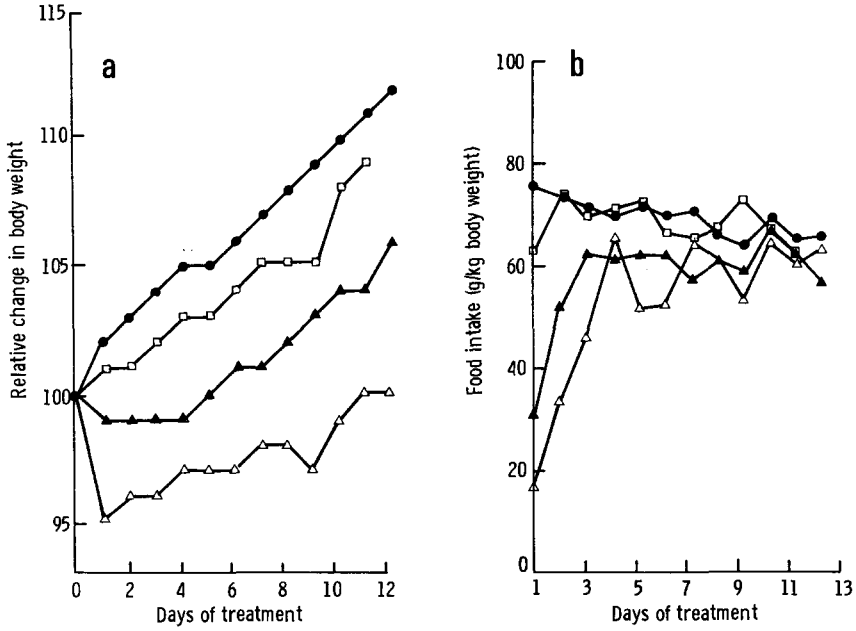

Fig. 1. Effects of D-fenfluramine on the growth rates and the food intake of rats. Rats were injected intraperitoneally with 2.5 mg/kg of D-fenfluramine twice a day (□; 12 rats), or once a day with 10 mg/kg (▲; 24 rats), or 25 mg/kg (△; 12 rats). Forty-eight control rats (●) were injected with the equivalent volume of 0.16 M NaCl (see Materials and Methods section). The average growth rates (a) were calculated relative to the weight of each rat which was taken immediately before the first injection. The rats were housed four to a cage. The food intakes (b) are the averages for the cages for each group and they are expressed relative to the weight of the rats in each cage. The corn oil diet had an estimated metabolizable energy content of 17.1 MJ/kg [35]. Error bars have been omitted for the sake of simplicity.

(Table 1) to gain an impression of whether the treatment with D-fenfluramine had altered the fat content of the body. Increasing the dose of D-fenfluramine progressively decreased the relative weight of the fat pads.

Effects of D-fenfluramine on the concentrations of circulating corticosterone, fatty acid, glucose, triacylglycerol and glycerol in response to a fructose load. The chronic treatment of rats with 10 mg of D-fenfluramine per kg did not significantly modify the initial increase in corticosterone concentrations, but it did decrease the duration of the response (Fig. 2a). This effect was also seen at the 25 mg/kg dose but not at the dose of 2.5 mg of D-fenfluramine per kg twice a day (Table 2).

Table 1. Effect of various doses of D-fenfluramine on the weights of epididymal fat pads in rats

Treatment	Fat pad wt / Body wt × 100
Control	1.21 ± 0.19 (47)
2.5 mg of fenfluramine twice a day	1.13 ± 0.10 (11)
10 mg of fenfluramine once a day	1.00 ± 0.16 (24)*
25 mg of fenfluramine once a day	0.88 ± 0.17 (12)*

Rats were treated with D-fenfluramine for the time indicated in Fig. 1 and the weight of the fat pads was determined at the time when they were killed. Results are means ± S.D. (N).

* Indicates a significant difference from the control of $P < 0.001$.

The two higher doses of D-fenfluramine also decrease the rise in circulating fatty acids (Fig. 2b, Table 2), but there were no significant differences for the lowest dose of the drug (Table 2). Evidence was also provided that all three doses of D-fenfluramine decreased the concentration of circulating glycerol (Fig. 2, Table 2).

Treatment of the rats with D-fenfluramine did not appear to alter the concentration of serum glucose relative to that of the control rats at any of the doses that were employed (Fig. 2, Table 2).

There was, however, an effect on the concentrations of circulating triacylglycerols. The initial concentration of circulating triacylglycerol before the fructose load was significantly lower in the rats treated with 10 mg of fenfluramine per kg (Fig. 2d). Similar hypotriglyceridaemic effects of fenfluramine have been reported previously. They appear to result from an inhibition of hepatic triacylglycerol synthesis and VLDL secretion [see ref. 2 for details]. The fructose load initially decreased the triacylglycerol concentration and then increased it (Fig. 2d) as reported previously [33]. The subsequent increase in the concentration of triacylglycerol was less than for the controls for all of the groups of rats that were treated with D-fenfluramine (Fig. 2d, Table 2).

DISCUSSION

Treatment of the rats with 10 or 25 mg of D-fenfluramine per kg produced a loss of body weight

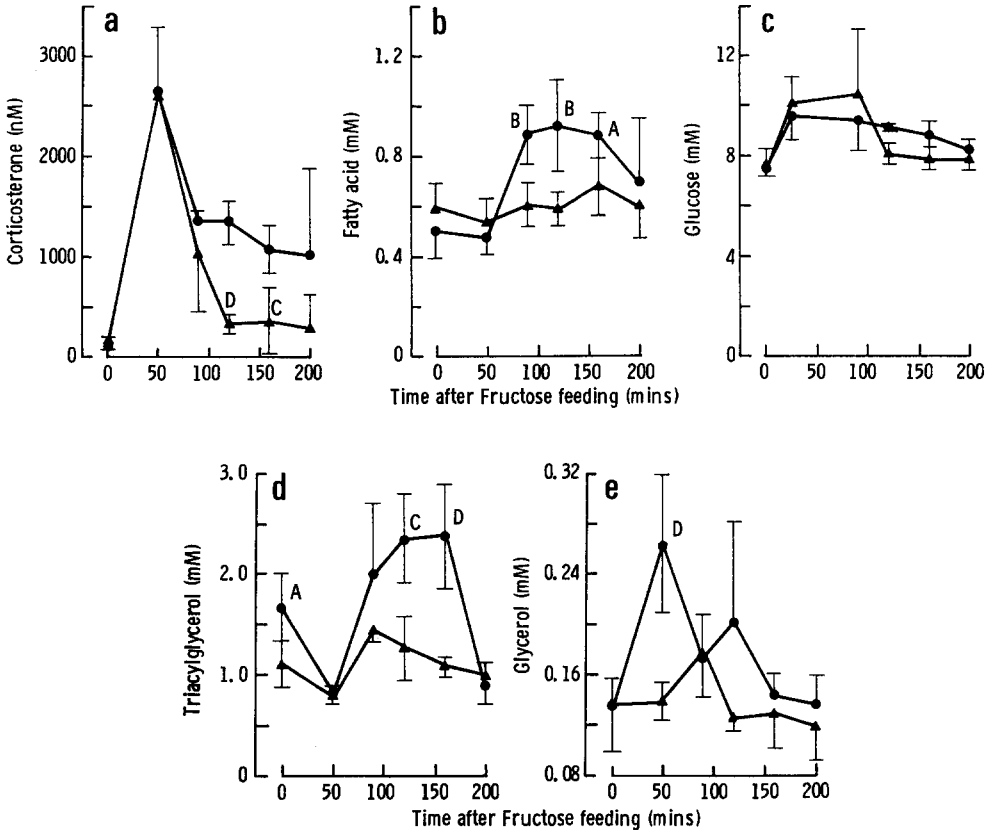

Fig. 2. Effect of D-fenfluramine on the serum concentrations of (a) corticosterone, (b) fatty acid, (c) glucose, (d) triacylglycerols and (e) glycerol. Rats were injected once a day for 12 consecutive days with 10 mg of D-fenfluramine/kg (▲) or with 0.16 M NaCl (●). Two hours after the last injection they were fed by stomach tube with 9.5 g of fructose/kg and they were killed at the times indicated. Results are expressed as means ± S.D. for four rats per group, except for the control rats at 90 min where these were results from three rats. The significance of the difference between the control and fenfluramine groups which were treated within 8 min of each other were calculated by using an unpaired t test and they are indicated as follows: A, $P < 0.05$; B, $P < 0.02$; C, $P < 0.01$; and D, $P < 0.005$.

and an anorectic effect during the first day of treatment. Food intake then approached that of the control rats and after about 4 days of daily treatment the weight gains of the rats treated with 10 mg of D-fenfluramine/kg paralleled that of the controls (Fig. 1). Similar effects have been observed by other investigators using longer treatment periods and a dose of 20 mg of fenfluramine/kg [38]. The rats treated with the higher dose of 25 mg of D-fenfluramine/kg gained weight from days 2 to 12 of treatment. However, the weight gain over this limited period appeared to be slightly less than for the control rats (Fig. 1a).

It has been suggested that the animals become rapidly tolerant to fenfluramine since food intake and weight gain is rapidly restored. However, if D-fenfluramine were simply acting by decreasing food intake then the rats should have regained their loss of weight and the growth curves should have converged with that of the control rats. This is in fact seen when the fenfluramine treatment is stopped [38, 39]. It has been proposed that fenfluramine persistently lowers the body weight set-point, and that anorexia is secondary to the weight suppressing effect [38–40]. The return to normal appetite as expressed in absolute terms [38], or relative to body weight (Fig. 1b) after repeated dosing with fenfluramine, was not caused by pharmacological tolerance. It presumably resulted from a physiological and behavioural adaptation to a lowered body weight [38, 39]. The lower body weight in the present animals was also accompanied by a decrease in the relative weight of the epididymal fat pad (Table 1).

The effects of D-fenfluramine and its metabolite D-norfenfluramine on feeding behaviour are thought to be mediated through their effects on 5-hydroxytryptamine (5-HT) metabolism [41, 42]. D-Fenfluramine probably acts by releasing 5-HT from nerve endings, whereas D-norfenfluramine may have a direct action on postsynaptic serotonin receptors. Both of these effects acutely depress food intake [41]. In the present experiments D-fenfluramine was injected at the beginning of the light period. Although D-fenfluramine has a $t_{\frac{1}{2}}$ of about 2.6 hr in the rat, it is converted to D-norfenfluramine which has a $t_{\frac{1}{2}}$ of approx. 12.5 hr [43]. This means that the latter compound would still have been available at the beginning of the dark period to modify feeding behaviour when most of the food is normally consumed by rats.

Table 2. Effect of chronic administration of high and low doses of D-fenfluramine on the concentrations of circulating corticosterone, fatty acids, glucose, triacylglycerols and glycerol in rats

Treatment	Concentration in serum	Time after fructose feeding (min)	Control	Fenfluramine treated	Significance
(A) 25 mg of fenfluramine/kg once a day for 12 days	Corticosterone (nM)	90	1110 ± 147 (4)	645 ± 71 (4)	P <0.005
		120	1082 ± 253 (4)	380 ± 263 (4)	P <0.05
		160	996 ± 167 (3)	530 ± 183 (4)	P <0.025
	Fatty acids (μM)	90	821 ± 243 (4)	578 ± 97 (4)	n.s.
		120	851 ± 146 (4)	730 ± 110 (4)	n.s.
		160	786 ± 120 (4)	604 ± 70 (4)	P <0.05
	Triacylglycerols (mM)	90	1.77 ± 0.46 (4)	0.90 ± 0.10 (4)	P <0.02
		120	1.36 ± 0.22 (4)	0.81 ± 0.22 (4)	P <0.02
		160	1.31 ± 0.17 (3)	0.78 ± 0.29 (4)	P <0.05
	Glycerol (μM)	90	212 ± 27 (4)	172 ± 73 (4)	n.s.
		120	223 ± 62 (4)	109 ± 27 (4)	P <0.02
		160	166 ± 22 (3)	142 ± 22 (4)	n.s.
(B) 2.5 mg of fenfluramine/kg twice a day for 11 days	Triacylglycerol (mM)	50	0.80 ± 0.08 (4)	0.53 ± 0.08 (4)	P <0.005
		90	1.96 ± 0.48 (4)	1.18 ± 0.32 (4)	P <0.05
		120	1.55 ± 0.42 (4)	0.98 ± 0.33 (3)	n.s.
	Glycerol (μM)	50	164 ± 21 (4)	148 ± 22 (4)	n.s.
		90	168 ± 4 (4)	133 ± 27 (4)	P <0.05
		120	171 ± 18 (4)	176 ± 27 (3)	n.s.

Results are expressed as means ± S.D. (N) and the significance of the difference between control and treated rats is calculated as described in Fig. 2. n.s. = not significant. There were no significant differences from the controls for the concentrations of glucose for the rats treated with 25 mg/kg of D-denfluramine, or for corticosterone, fatty acids and glucose at the 2.5 mg/kg dose.

The other experiments that are reported in this paper were performed to determine whether long-term treatment with D-fenfluramine would produce persistent changes in metabolism at a time when the food intakes and the rates of weight gain of the rats were similar to the controls. This was particularly important since it was necessary to dissociate these metabolic changes from the immediate effects of food deprivation which in itself will modify the metabolism of corticosterone, glucose and triacylglycerol. Furthermore, the metabolic measurements were made after feeding a standard meal of fructose to all groups of rats.

In response to this metabolic load the control rats exhibited a stress response as shown by the prolonged release of corticosterone and the increase in circulating concentrations of glycerol and fatty acids (Fig. 2). This stress response was deliberately exaggerated by maintaining the rats on a diet rich in corn oil [33]. The duration of this corticosterone response was decreased in those rats that were treated with the 10 and 25 mg/kg doses of D-fenfluramine (Fig. 2, Table 2) which also significantly decreased body weight (Fig. 1) and the relative weight of the epididymal fat pad (Table 1). The corticosterone response in these animals more closely resembles that expected from rats fed on a high carbohydrate diet [33]. The higher doses of D-fenfluramine also decreased the rise in circulating fatty acid and glycerol (Fig. 2, Table 2). The lowest dose of D-fenfluramine that had little effect on initial food intake and the weight gain of the rats (Fig. 1) had no significant effect on the duration of the corticosterone response after feeding fructose (Table 2). There was also no significant decrease in the serum fatty acid concentrations, but there was an indication of a significant decrease in circulating glycerol.

It is therefore difficult to be certain whether there is always a direct relation between the action of fenfluramine in decreasing a stress response and lipolysis, and therefore preventing the increased recirculation of fatty acids from the liver as triacylglycerols. The concentrations of fatty acids and glycerol in the blood are only indirect measurements of lipolysis since they indicate the balance between release into the blood and uptake by other tissues. Corticosterone was used as a hormonal indicator of stress rather than the catecholamines which change very rapidly in concentration even when handling the rats. It is not suggested that the direct effects of corticosterone in stimulating the synthesis of hepatic triacylglycerols would have been observed in the present experiments. For instance, the glucocorticoid-induced increase in phosphatidate phosphohydrolase activity takes about 4 hr before it becomes evident [44]. The long-term effect of a fenfluramine-like drug in partially preventing the corticosterone-induced increase in the phoshohydrolase activity has already been reported [21, 23]. Fenfluramine can, however, inhibit triacylglycerol synthesis directly by inhibiting the phosphohydrolase activity [see ref. 2 for details] and this could provide a further explanation for the observed lowering of circulating triacylglycerols in the present experiments. It should be noted that these measurements were performed between 120 and 420 min after the injection of D-fenfluramine when significant concentrations of the drug and its metabolites should have been present in the blood [30, 43].

By contrast to the effect on the concentration of circulating triacylglycerols fenfluramine did not

significantly alter the concentration of glucose in the blood after the fructose load test (Fig. 2, Table 2). However, a related drug, benfluorex, did have this effect (D. N. Brindley, unpublished work).

The chronic effect of D-fenfluramine in decreasing the concentrations of corticosterone after the fructose test contrasts with its actions when administered acutely. In the latter case fenfluramine [31, 32] rapidly increases the concentration of circulating corticosterone and produces a stress response [1, 2]. This occurs when there is a marked anorectic effect and weight loss (Fig. 1). The corticosterone release is thought to be initiated by the release of 5-HT from the hypothalamus [31, 32] which in turn causes the production of corticoliberin and thereafter corticotropin. After chronic administration D-fenfluramine does not significantly increase the concentration of circulating corticosterone and there is no evidence for an increase in lipolysis (Fig. 2). Thus, with acute and chronic fenfluramine-treatment there seems to be an inverse relation between food intake and weight gain, and the release of corticosterone in response to a stress stimulus. This link may be provided by changes in 5-HT metabolism [2]. Alternatively, the altered body weight and fat content (Fig. 1, Table 1), and presumably the change in body weight set-point that is caused by D-fenfluramine [38–40] could be associated with a decrease in stress response. There was in fact a significant correlation of $P < 0.05$ between the relative weights of the fat pads and the corticosterone concentrations for all of the rats that were killed 90 min after feeding with fructose. This value was obtained from a Spearman Rank Correlation analysis using a two-tailed test. The equivalent level of significance for the rats killed 120 min after fructose feeding was $P < 0.02$.

The present work provides further evidence that animals do not become tolerant to anorectic drugs as has often been supposed. In particular, treating rats chronically with D-fenfluramine produces a persistent hypotriglyceridaemic effect (Fig. 2). The higher doses of D-fenfluramine decreased body and epidiymal fat pad weights and also lowered the stress and lipolytic responses after a fructose load test. In terms of relating these results to the treatment of obesity, it must be emphasised that the present work was performed with young rats that were growing rapidly and which were not obese. However, if similar persistent changes in metabolism occur in adult human beings then this could further justify the long-term use of D-fenfluramine in treating obesity.

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REFERENCES

1. W. N. Dannenburg, in *Biochemical Pharmacology of Obesity* (Ed. P. B. Curtis-Prior), p. 263. Elsevier Science Publishers, Amsterdam (1983).
2. D. N. Brindley, in *Biochemical Pharmacology of Obesity* (Ed. P. B. Curtis-Prior), p. 285. Elsevier Science Publishers, Amsterdam (1983).
3. J. M. Amantruda, S. A. Danahy and C. L. Chang, *Biochem. J.* **212**, 135 (1983).
4. D. S. Robinson, S. M. Parkin, B. K. Speake and J. A. Little, in *The Adipocyte and Obesity: Cellular and Molecular Mechanisms* (Eds. A. Angel, C. H. Hollenberg and D. A. K. Roncari), p. 127. Raven Press, New York (1983).
5. D. N. Brindley and R. G. Sturton, in *Phospholipids* (Eds. J. N. Hawthorne and G. B. Ansell), p. 179. Elsevier Biomedical Press, Amsterdam (1982).
6. D. N. Brindley and N. Lawson, in *The Adipocyte and Obesity: Cellular and Molecular Mechanisms* (Eds. A. Angel, C. H. Hollenberg and D. A. K. Roncari), p. 155. Raven Press, New York (1983).
7. R. A. Pittner, R. Fears and D. N. Brindley, *Biochem. J.* **225**, 455 (1985).
8. C. Cascales, E. H. Mangiapane and D. N. Brindley, *Biochem. J.* **219**, 911 (1984).
9. S. C. Butterwith, A. Martin and D. N. Brindley, *Biochem. J.* **222**, 487 (1984).
10. P. Martin-Sanz, R. Hopewell and D. N. Brindley, *FEBS Lett.* **175**, 284 (1985).
11. D. C. Cameron, W. P. U. Kennedy, P. D. Samuel and G. K. Crompton, *Curr. Med. Res. Opin.* **1**, 153 (1972).
12. S. A. Tomlinson, J. G. Lines and M. A. M. Greenfield, *Postgrad. Med. J.* **51** (Suppl. 1) 166 (1975).
13. S. A. Antelman, A. R. Caggiula, A. J. Eichler and R. R. Lucik, *Curr. Med. Res. Opin.* **6** (Suppl. 1), 73 (1979).
14. F. Borsini, C. Bendotti, B. Przewlocka and R. Samanin, *Eur. J. Pharmac.* **94**, 109 (1983).
15. A. Odomoso and C. W. M. Wilson, *Br. J. Pharmac.* **48**, 326P (1973).
16. A. Odomoso and C. W. M. Wilson, *Int. J. Obesity* **3**, 123 (1979).
17. J. R. Hodges and R. J. Holston, *Br. J. Pharmac.* **40**, 740 (1970).
18. M. K. Fordyce and M. E. Kassouny, *J. Nutr.* **107**, 1846 (1977).
19. I. Bjorkhem, A. Kallner and K-E. Karlman, *J. Lipid Res.* **19**, 695 (1978).
20. E. Ginter, *Adv. Lipid Res.* **16**, 167 (1978).
21. D. N. Brindley, R. G. Sturton, P. H. Pritchard, J. Cooling and S. L. Burditt, *Curr. Med. Res. Opin.* **6** (Suppl. 1), 91 (1979).
22. P. H. Pritchard, J. Cooling, S. L. Burditt and D. N. Brindley, *J. Pharm. Pharmac.* **31**, 406 (1979).
23. P. H. Pritchard, M. Bowley, S. L. Burditt, J. Cooling, H. P. Glenny, S. Pawson, R. G. Sturton and D. N. Brindley, *Biochem. J.* **166**, 639 (1977).
24. P. H. Pritchard and D. N. Brindley, *J. Pharm. Pharmac.* **29**, 343 (1977).
25. R. T. Jung, R. G. Campbell, W. P. T. James and B. A. Callingham, *Lancet* **i**, 1043 (1982).
26. M. O. Carruba and P. Mantegazza, *Int. Congr. on Obesity* (New York) **10A** (1983).
27. G. A. Bray and D. A. York, *Physiol. Rev.* **59**, 719 (1979).
28. B. M. King, A. R. Banta, L. N. Tharel, B. K. Bruce and L. A. Frohman, *Am. J. Physiol.* **245**, E194 (1983).
29. J. C. Le Douarec, H. Schmitt and M. Laubie, *Arch. Int. Pharmacodyn.* **161**, 206 (1966).
30. S. Garratini, S. Caccia, T. Mennini, R. Samanin, S. Consolo and H. Ladinsky, *Curr. Med. Res. Opin.* **6**, (Suppl. 1) 15 (1979).
31. G. Schettini, A. Quattrone, G. F. Di Renzo and P. Preziosi, *Pharmac. Res. Commun.* **6**, 545 (1979).
32. R. W. Fuller, H. D. Snoddy and J. A. Clemens, *Pharmac. Res. Commun.* **13**, 275 (1981).
33. D. N. Brindley, J. Cooling, H. P. Glenny, S. L. Burditt and I. S. McKechnie, *Biochem. J.* **200**, 275 (1981).
34. D. N. Brindley, J. Cooling, S. L. Burditt, P. H. Pritchard, S. Pawson and R. G. Sturton, *Biochem. J.* **180**, 195 (1979).
35. N. Lawson, R. J. Jennings, A. D. Pollard, R. G.

Sturton, S. J. Ralph, C. A. Marsden, R. Fears and D. N. Brindley, *Biochem. J.* **200**, 265 (1981).
36. N. Lawson, A. D. Pollard, R. J. Jennings, M. I. Gurr and D. N. Brindley, *Biochem. J.* **200**, 285 (1981).
37. C. Cousins, C. A. Marsden and D. N. Brindley, *Biochem. J.* **206**, 431 (1982).
38. D. A. Levitsky, B. J. Strupp and J. Lupoli, *Pharmac. Biochem. Behav.* **14**, 661 (1981).
39. A. J. Stunkard, *Life Sci.* **30**, 2043 (1982).
40. M. Fantino, F. Faicon and Y. Rolland, *Int. Congr. on Obesity* (New York) **35A** (1983).
41. F. Borsini, C. Bendotti, A. Aleotti, R. Samanin and S. Garattini, *Pharmac. Res. Commun.* **14**, 671 (1982).
42. R. Samanin, in *Biochemical Pharmacology of Obesity* (Ed. P. B. Curtis-Prior), p. 339. Elsevier Science Publishers, Amsterdam (1983).
43. S. Caccia, M. Ballabio, G. Guiso, M. Rochetti and S. Garattini, *Arch. int. Pharmacodyn.* **258**, 15 (1982).
44. R. J. Jennings, N. Lawson, R. Fears and D. N. Brindley, *FEBS Lett.* **133**, 119 (1981).