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COMPARATIVE EFFECTS OF AMPHETAMINE AND FENFLURAMINE ON LIPID BIOSYNTHESIS AND ABSORPTION IN THE RAT

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Abstract—The effects of amphetamine and fenfluramine on lipid biosynthesis and intestinal absorption of triglycerides in the rat were observed independently of the anorectic activity of these drugs. Amphetamine and fenfluramine significantly reduced *de novo* lipogenesis and cholesterogenesis from ^3H_2O and [^{14}C] alanine in isolated hepatocytes. Fenfluramine was four times more potent an inhibitor than amphetamine. At high concentrations (above 2 mM), fenfluramine inhibited $^{14}CO_2$ production from [^{14}C] alanine in isolated hepatocytes while amphetamine stimulated $^{14}CO_2$ production at all concentrations tested (0.25 to 8 mM). Lipogenesis in liver and adipose tissue was not reduced *in vivo* by the acute administration of amphetamine. It, however, reduced lipogenesis in the small intestine. Fenfluramine significantly depressed *in vivo* lipogenesis in liver, small intestine and adipose tissue at 10 and 20 mg/kg, i.p. Hepatic cholesterogenesis was depressed significantly *in vivo* by both drugs. Fenfluramine increased serum free fatty acids while amphetamine produced no change. Triglycerides were increased significantly by fenfluramine only. Serum cholesterol and phospholipids were unchanged. Fenfluramine at 40 and 60 mg/kg, p.o., diminished significantly the intestinal absorption of triglycerides and depressed the accumulation of lipid in the liver. Analogous effects were not observed with amphetamine. Amphetamine was a weak inhibitor of rat pancreatic lipase (EC 3.1.1.3) (K_i = 21 mM) and fenfluramine was a stronger inhibitor (K_i = 3.3 mM).

The anorectic effect of compounds of the phenethylamine class has been recognized for almost 40 years [1]. Amphetamine and especially fenfluramine [2] have been implicated as agents affecting carbohydrate and lipid metabolism, indicating that peripheral actions of these antiobesity drugs might be important. Fenfluramine, but not amphetamine, increased glucose uptake by rat hemidiaphragm and human gluteus muscle preparations [3]. The increase in glucose uptake appeared to be linked with the action of insulin, and hypoglycemic responses to fenfluramine have been reported in normal, diabetic and obese subjects [4, 5].

Amphetamine and fenfluramine have been reported to affect lipid metabolism by decreasing the postprandial rise in plasma triglycerides in corn oil-loaded rats [6, 7] and elevating plasma free fatty acids in rats [8, 9]. Fenfluramine reduced serum triglycerides and increased free fatty acids in man [8]. However, it was difficult to assess whether these changes resulted from weight reduction or reduced food intake or from a biochemical action of the drug. More recent studies have indicated that amphetamine and fenfluramine inhibit *in vitro* enzymes involved in lipid metabolism [10–15]. Brindley and Bowley [10] reported that fenfluramine (0.8 mM) inhibited rat liver phosphatidate phosphohydrolase (EC 3.1.3.4) and was 4- to 8-fold more effective than amphetamine. Fenfluramine (10^{-3} M) was reported by Wilson and Galton [11] to inhibit the incorporation of palmitate and glucose into neutral lipid of isolated human adipose tissue and tentatively identified the site of inhibition as the acyl transfer reaction of triglyceride synthesis. Additionally, norfenfluramine, an active metabolite of fenfluramine, has been reported to inhibit *in vitro* lipogenesis and CO_2 production in isolated human adipose tissue [12]. Fenfluramine appeared to reduce intestinal motility [7] and interfere with post-absorptive re-esterification by inhibition of rat intestinal palmitoyl CoA:monoolein transferase (EC 2.3.1.–) [13]. Furthermore, fenfluramine [14] and an analog, 780 SE [15], have been reported to inhibit pancreatic lipase. However, a study of healthy humans revealed that neither fenfluramine nor 780 SE altered fat absorption [16].

Current literature does not indicate whether amphetamine or fenfluramine affects *de novo* lipogenesis or cholesterogenesis *in vivo*. The present studies were designed to elucidate the effects of amphetamine and fenfluramine on lipid biosynthesis and intestinal absorption in the rat independently of the anorectic activity produced by these drugs. *De novo* hepatic, intestinal and adipose lipogenesis and hepatic cholesterogenesis from ^3H_2O and [^{14}C] alanine and dietary lipid absorption were determined *in vivo* and *in vitro*.

MATERIALS AND METHODS

Animals and dietary treatment. Female rats (Charles River CD strain, 180–200 g) were housed individually in wire bottom cages in a temperature (22°) and light regulated [12 hr light (6:00 a.m. to 6:00 p.m.) and 12 hr dark] room. They had free access to a commerical diet

(Purina Rodent Chow, Ralston Purina Co., St. Louis, MO) and water for a minimum of 1 week prior to experiments.

In the lipogenesis and cholesterogenesis experiments the rats were fasted 24 hr, then meal-fed a G-70 (70% glucose) diet (Bio-Serv. Inc., Frenchtown, NJ) from 8:00 to 11:00 daily for 9 days. Previous investigations have demonstrated that the above regimen elevated hepatic rates of fatty acid and cholesterol synthesis *in vivo* [17, 18].

Preparation of hepatocytes, lipid synthesis and CO_2 production. Hepatocytes were prepared from meal-fed Charles River rats as previously described [18]. Briefly, rats were anesthetized with Nembutal, livers were perfused *in situ* and hepatocytes were isolated in Krebs–Henseleit bicarbonate buffer, pH 7.4. Incubations with isolated hepatocytes were performed in triplicate at 37°. Each incubation flask contained, in a total volume of 2.1 ml, 10.5 mg cells (dry weight), 1 ml of Krebs–Henseleit buffer, pH 7.4, radioactive substrate (1 μCi [U-^{14}C]alanine, 1 mCi ^3H_2O and 1 μmole alanine) and 0.3% glucose. The appropriate concentration of either amphetamine or fenfluramine was prepared in H_2O, pH 7.4. ^3H_2O was used to determine the total rate of lipogenesis and cholesterogenesis independently of carbon precursors of acetyl CoA; [^{14}C]alanine was utilized as an acetyl CoA precursor. After incubation, the $^{14}CO_2$ produced was collected in an ethanolamine trap based on the method of Ontko [19]. The $^{14}CO_2$–ethanolamine mixture was transfered to a scintillation vial containing 2,5-bis(5′-tert.-butyl-2-benzoxazolyl)-thiophene (BBOT) scintillation fluid (Yorktown Research, South Hackensack, NJ). Rates of lipogenesis and cholesterogenesis were determined as described previously [18]. Data are expressed as nmoles ^3H_2O or [^{14}C]alanine converted into fatty acids or cholesterol/mg dry weight cells/60 min.

In vivo *lipogenesis and cholesterogenesis.* Amphetamine or fenfluramine was administered (intraperitoneally) 30 min after the 3-hr G-70 meal. One hr later the radioactive pulse consisting of 1 mCi ^3H_2O, 5 μCi [U-^{14}C]alanine, 12.3 mg alanine and 30.6 mg α-ketoglutarate (an amine acceptor for transaminase) was administered. The rats were killed 30 min later by decapitation. Blood was collected, allowed to clot on ice for 30 min, and centrifuged. Livers, small intestines and retroperitoneal adipose tissue were excised immediately and placed on ice. Intestines were freed of fat and rinsed repeatedly with saline. Fatty acid and cholesterol synthesis were determined as described previously [18]. Data are expressed as μmoles ^3H_2O or nmoles [^{14}C]alanine converted to fatty acids or cholesterol/g of tissue/30 min.

Serum analyses. Serum was analyzed for triglycerides and cholesterol by enzymatic procedures [20, 21]. Free fatty acids [22] and phospholipids [23] were determined also.

In vivo *absorption of triglycerides.* The effectiveness of amphetamine or fenfluramine in reducing the intestinal absorption of triglycerides was evaluated in two types of experiments. In the first experiment, amphetamine or fenfluramine was administered by gavage in 1% gum arabic 15 min prior to corn oil administration (20 ml/kg). Control rats received 1% gum arabic. Blood samples were taken from the tails at 0, 2, 4, 6, 8, 10 and 12 hr in heparinized microcentrifuge tubes. Plasma triglycerides were determined as above [20]. In the second experiment, radioactive corn oil (0.33 μCi glycerol tri[1-^{14}C] oleate/ml of corn oil, 20 ml/kg) was administered to rats by gavage 15 min after receiving either amphetamine or fenfluramine orally. After 10 and 12 hr the rats were killed by decapitation, blood was collected and livers were excised and chilled on ice. Serum was analyzed for radioactivity by counting in a BBOT scintillation mixture. Liver lipids were extracted using the method of Folch *et al.* [24] and radioactive lipids were determined by counting in a toluene based PPO–POPOP* scintillation mixture (LSC Complete, Yorktown Research, South Hackensack NJ). Data are expressed as μmoles glycerol tri[1-^{14}C] oleate/g of liver or per ml of serum based on the specific activity of the original corn oil.

Preparation and assay of pancreatic lipase. Pancreatic lipase was prepared from rats as described previously [25] by adapting the method of Scheele and Palade [26] for the guinea pig pancreas. Pancreatic lipase activity was determined by the titrimetric assay described by Maylie *et al.* [27]. An olive oil (6 or 28 mM)–sodium taurocholate (4 mM) stabilized emulsion was used as the substrate. The assay was initiated by the addition of lipase. The long-chain fatty acids released at 25°, pH 8, were titrated continuously by a recording pH Stat (Radiometer, Copenhagen). The lipase activity was determined directly from the slope of the linear portion of the curve. Amphetamine or fenfluramine was added to the assays (10–200 μl) in 90% ethanol; 200 μl of 90% ethanol produced no effect on pancreatic lipase activity as assayed by the above method. Inhibition constants were determined by the graphical method of Dixon and Webb [28].

Materials. ^3H_2O (1 Ci/ml) and [U-^{14}C]alanine (165 mCi/m-mole) were purchased from New England Nuclear, Boston, MA; glycerol tri[1-^{14}C]oleate (57.5 mCi/m-mole) from Amersham/Searle, Des Plaines. IL; the cholesterol and glucose test kits from Abbott Labs., Pasadena, CA; and the triglyceride test kit from CalBiochem, La Jolla, CA. Amphetamine sulfate was kindly provided by Smith, Kline & French Labs., Philadelphia, PA. Fenfluramine was kindly provided by the A. H. Robbins Co., Richmond, VA. Corn oil (Mazola) was purchased locally. All other chemicals were purchased from Sigma Chemical Co., St. Louis, MO, and were of the highest quality available.

Statistical methods. All experiments were performed at least twice. Data were processed for outliers [29]. A two-sided *t*-test was used to evaluate all experimental results [30]. Areas under the absorption curves were determined using a computer program based on the method of Grubbs [31].

RESULTS

Effect of amphetamine and fenfluramine on fatty acid and cholesterol syntheses and CO_2 production in isolated hepatocytes. The effects of amphetamine and

*PPO = 2,5-diphenyloxazole; POPOP = 1,4-bis-2-(5-phenyloxazolyl)-benzene.

Table 1. Effect of amphetamine and fenfluramine on fatty acid and cholesterol syntheses and CO_2 production in isolated hepatocytes

Compound	Concn (mM)	Fatty acid synthesis*		Cholesterol synthesis*		CO_2 production*
		^3H_2O	[^{14}C]alanine	^3H_2O	[^{14}C]alanine	[^{14}C]alanine
Control		48.0 ± 2.8	2.6 ± 0.2	9.3 ± 0.2	0.51 ± 0.00	27.2 ± 0.4
Amphetamine	8.0	22.4 ± 3.3†	1.3 ± 0.2†	1.9 ± 0.1†	0.08 ± 0.00†	36.6 ± 0.9†
	4.0	44.4 ± 3.7	2.9 ± 0.2	4.6 ± 0.3†	0.28 ± 0.01†	36.2 ± 2.1†
	2.0	50.8 ± 2.5	3.6 ± 0.3	7.0 ± 0.2†	0.47 ± 0.02	35.5 ± 1.0†
	1.0	63.4 ± 2.0†	4.4 ± 0.2†	8.9 ± 0.3	0.54 ± 0.01†	33.9 ± 0.9†
	0.5	78.7 ± 7.5†	5.1 ± 0.3†	9.4 ± 0.3	0.59 ± 0.01†	31.7 ± 0.4†
	0.25	70.7 ± 7.1†	4.2 ± 0.3†	10.0 ± 0.2	0.57 ± 0.01†	30.0 ± 1.1†
Fenfluramine	8.0	1.5 ± 0.2†	0.01 ± 0.00†	0.2 ± 0.0†	0.00 ± 0.00†	6.2 ± 0.3†
	4.0	7.1 ± 0.8†	0.4 ± 0.0†	0.7 ± 0.0†	0.00 ± 0.00†	14.6 ± 1.1†
	2.0	17.3 ± 1.2†	0.8 ± 0.1†	1.0 ± 0.0†	0.03 ± 0.00†	23.5 ± 0.9†
	1.0	48.8 ±	2.5 ± 0.3	2.8 ± 0.1†	0.15 ± 0.01†	26.2 ± 1.4
	0.5	54.5 ± 10.9	3.2 ± 0.6	5.5 ± 0.1†	0.37 ± 0.01†	29.8 ± 0.7
	0.25	60.1 ± 1.0†	3.7 ± 0.2†	7.0 ± 0.4†	0.42 ± 0.01†	29.0 ± 1.4

*Expressed as nmoles ^3H_2O or [^{14}C]alanine converted/mg dry cell weight/60 min. Data are expressed as the mean ± S.E. of triplicate incubations.

†Significantly different from control ($P \leq 0.05$).

fenfluramine on fatty acid and cholesterol syntheses and CO_2 production in isolated rat hepatocytes are presented in Table 1. Amphetamine at low concentrations stimulated significantly fatty acid synthesis from both ^3H_2O (132–164 per cent) and [^{14}C]alanine (162–198 per cent). Inhibition (53 per cent) was observed only at 8 mM. Fenfluramine was observed to stimulate fatty acid synthesis significantly only at 0.25 mM. At concentrations above 1 mM, fenfluramine inhibited fatty acid synthesis significantly. Fenfluramine at 2, 4 and 8 mM produced 64, 85 and 97 per cent inhibition of fatty acid synthesis from ^3H_2O. These same concentrations inhibited fatty acid synthesis from [^{14}C]alanine by 70, 90 and 100 per cent respectively.

Amphetamine inhibited cholesterogenesis from ^3H_2O and [^{14}C]alanine (Table 1). Concentrations of 2, 4, and 8 mM amphetamine reduced significantly cholesterol synthesis from ^3H_2O by 24, 50 and 80 per cent respectively. These same concentrations produced inhibitions from [^{14}C]alanine of 10, 45 and 85 per cent respectively. Concentrations of amphetamine at and below 1 mM were not effective. Fenfluramine at all concentrations (0.25 to 8 mM) significantly inhibited cholesterogenesis from both ^3H_2O and [^{14}C]alanine in a dose–dependent manner. Cholesterogenesis from ^3H_2O was inhibited 25–98 per cent and from [^{14}C]alanine 18–100 per cent. CO_2 production from [^{14}C]alanine was stimulated significantly by all concentrations of amphetamine (0.25 to 8 mM). In contrast, fenfluramine at 2 mM and above significantly reduced CO_2 production.

Effect of amphetamine and fenfluramine on rates of lipid biosynthesis in vivo. The effects of amphetamine and fenfluramine on rates of fatty acid and cholesterol syntheses *in vivo* were similar to those observed in isolated hepatocytes, and are presented in Tables 2 and 3. Fatty acid synthesis in liver and adipose tissue was not inhibited significantly by intraperitoneal administration of 10 or 20 mg/kg of amphetamine (Table 2). Intestinal fatty acid synthesis from ^3H_2O and [^{14}C]alanine was inhibited significantly by

Table 2. Effect of amphetamine and fenfluramine on fatty acid synthesis *in vivo**

Compound	Concn (mg/kg, i.p.)	Fatty acid synthesis†					
		Liver		Intestine		Adipose	
		^3H_2O	[^{14}C]alanine	^3H_2O	[^{14}C]alanine	^3H_2O	[^{14}C]alanine
Control		48.2 ± 3.4	598 ± 43	6.2 ± 0.8	94.1 ± 8.5	153 ± 19	3376 ± 384
Amphetamine	10	41.6 ± 4.3	467 ± 64	4.3 ± 0.6	99.3 ± 14.0	149 ± 30	2740 ± 543
	20	44.5 ± 6.1	503 ± 79	3.8 ± 0.3‡	62.5 ± 4.5‡	100 ± 24	2408 ± 552
Fenfluramine	10	39.1 ± 4.3	462 ± 42‡	4.2 ± 0.4‡	72.1 ± 7.8‡	77 ± 16‡	1850 ± 361‡
	20	24.6 ± 2.2‡	314 ± 24‡	3.4 ± 0.4‡	59.3 ± 5.3‡	77 ± 15‡	1734 ± 386‡

*Rats were meal-fed for 3 hr. The indicated compound was administered (i.p.) 30 min later. After 60 min a ^3H_2O and [^{14}C]alanine pulse was administered (i.v.). Rats were killed 30 min later. Livers, small intestine and retroperitoneal adipose tissue were excised, saponified and analyzed for *de novo* lipogenesis. Data are expressed as the mean ± S.E. of two combined experiments with ten rats per experimental group, totaling twenty, except for adipose tissue where ten rats were used.

†Expressed as μmoles ^3H_2O or nmoles [^{14}C]alanine converted/g wet tissue/30 min.

‡Significantly different from control ($P \leq 0.05$).

Table 3. Effect of amphetamine and fenfluramine on hepatic cholesterol synthesis *in vivo**

Compound	Concn. (mg/kg, i.p.)	Cholesterol synthesis†	
		$[^3H]_2O$	[^{14}C]alanine
Control	10	0.57 ± 0.06	11.3 ± 0.07
Amphetamine	10	0.31 ± 0.03‡	8.1 ± 0.9‡
	20	0.35 ± 0.04‡	7.4 ± 0.6‡
Fenfluramine	10	0.32 ± 0.03‡	7.5 ± 0.8‡
	20	0.23 ± 0.04‡	6.7 ± 0.7‡

*Rats were meal-fed for 3 hr. The indicated compound was administered (i.p.) 30 min later. After 60 min a ^3H_2O and [^{14}C]alanine pulse was administered (i.v.). After 30 min the rats were killed and the livers were excised, saponified and analyzed for cholesterol. Data are expressed as the mean ± S. E. of two combined experiments with ten rats per experimental group, totaling twenty rats.

†Expressed as μmoles ^3H_2O or nmoles [^{14}C]alanine converted/g wet tissue/30 min.

‡Significantly different from control ($P \leq 0.05$).

amphetamine at 20 mg/kg. However, fenfluramine at both 10 and 20 mg/kg significantly depressed fatty acid synthesis in the three tissues examined. The inhibition was dose related in liver and intestine; approximately a 20–30 per cent inhibition was observed at 10 mg/kg and a 50 per cent inhibition at 20 mg/kg for both ^3H_2O and [^{14}C]alanine. Fenfluramine depressed fatty acid synthesis in adipose tissue by 50 per cent, independently of the dose.

Cholesterol synthesis in liver was depressed significantly by both amphetamine and fenfluramine (Table 3). A 46 and 38 per cent inhibition of cholesterol synthesis from ^3H_2O was produced by 10 and 20 mg/kg of amphetamine respectively. These same concentrations produced a 28 and 35 per cent inhibition of cholesterol synthesis from [^{14}C]alanine. The fenfluramine inhibition of cholesterol synthesis in liver at 10 and 20 mg/kg was 44 and 60 per cent, respectively, from ^3H_2O and 34 and 41 per cent, respectively, from [^{14}C]alanine.

Serum triglycerides were decreased significantly to 77 per cent of control at the 10 mg/kg dose of amphetamine (Table 4). The 20 mg/kg dose produced no effect. Fenfluramine at doses of 10 and 20 mg/kg produced significant increases in serum triglycerides, to 122 and 135 per cent of control. Fenfluramine at 20 mg/kg stimulated significantly free fatty acid levels (141 per cent of control). Serum free fatty acids were unchanged by treatment with amphetamine at either 10 or 20 mg/kg. There were no significant effects of amphetamine or fenfluramine on serum cholesterol or phospholipids.

Effect of amphetamine and fenfluramine on intestinal triglyceride absorption. Administration of amphetamine (15 mg/kg, p.o.) to rats resulted in a consistent and significant delay in the absorption of corn oil when compared to controls, as measured by the appearance of triglycerides in plasma (Fig. 1). The control group absorption peak occurred at 6 hr, then steadily declined. The amphetamine-treated group reached a serum trigylceride peak also at 6 hr; however, it was significantly lower than the control value and remained virtually unchanged for 12 hr. The area under the plasma triglyceride curve for the amphetamine-treated group (695 ± 200) was 74 per cent of the control (939 ± 227), which is not a significant difference. The shape of the amphetamine curve indicates a difference in the kinetics of appearance of triglycerides in the plasma but not in the total absorption of lipid. No steatorrhea was observed during the experiment.

Unlike amphetamine, fenfluramine at doses of 20 and 40 mg/kg did not delay the appearance of triglycerides in plasma, but depressed the peak, indicating a decrease in corn oil absorption (Fig. 2). However, a higher dose of fenfluramine, 60 mg/kg, appeared to cause both a delay and depression of corn oil absorption. The areas for control and 20, 40, and 60 mg/kg of fenfluramine are 2209 ± 603, 1724 ± 713, 1458 ± 123 and 973 ± 68 respectively. The latter two areas are significantly different from the control ($P \leq 0.05$). Steatorrhea was observed in the fenfluramine-treated rats.

To further understand the effects of amphetamine and fenfluramine on lipid absorption *in vivo*, rats were intubated with [^{14}C]triolein labeled corn oil after drug administration, and levels of serum triglycerides and of radioactivity in serum and liver lipids were determined at 10 and 12 hr (Fig. 3, panels A, B, and C). These two times were chosen because previous experiments indicated that significant lipid absorption would have occurred by 12 hr.

Table 4. Serum analyses*

Compound	Concn (mg/kg, i.p.)	Triglycerides (mg/100 ml)	Cholesterol (mg/100 ml)	Phospholipids (mg/100 ml)	Free fatty acids (m-equiv./liter)
Control		66 ± 4	75 ± 4	151 ± 10	243 ± 31
Amphetamine	10	51 ± 2†	71 ± 4	135 ± 4	236 ± 33
	20	71 ± 5	69 ± 4	151 ± 9	279 ± 40
Fenfluramine	10	80 ± 5†	67 ± 4	155 ± 7	251 ± 20
	20	89 ± 6†	77 ± 3	168 ± 7	343 ± 33†

*Results are expressed as mean ± S. E. of two combined experiments with ten rats per group per experiment, totaling twenty rats. Rats were meal-fed for 3 hr. The indicated compound was administered 30 min later (i.p.) and a ^3H_2O and [^{14}C]alanine pulse (i.v.) was given 60 min later. Rats were killed 30 min later and blood was collected on ice. Serum was obtained by centrifugation and analyzed.

†Significantly different from control ($P \leq 0.05$).

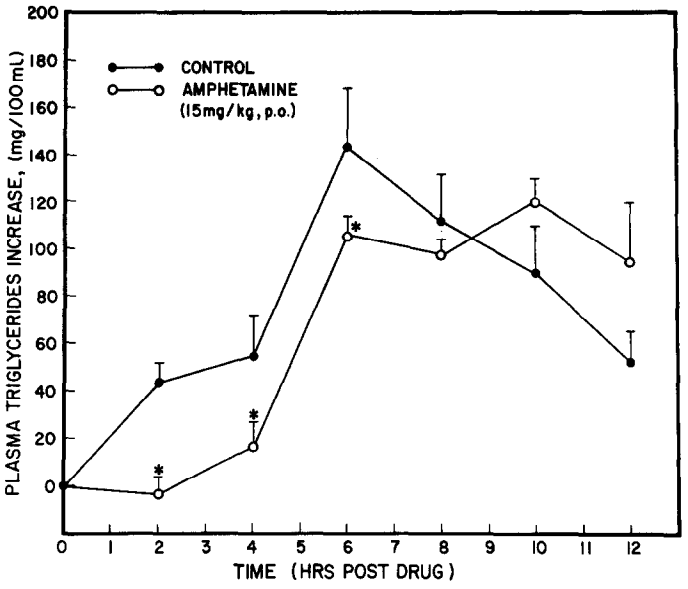

Fig. 1. Triglyceride absorption in amphetamine-treated rats. Amphetamine (15 mg/kg, p.o.) in 1% gum arabic (O-O), or 1% gum arabic alone (control) (●-●) was administered to each rat (ten per experimental group). After 15 min, corn oil (20 ml/kg, p.o.) was administered. Blood samples were taken from the tail at the indicated times in heparinized tubes. Plasmas were assayed for triglycerides [20]. Values are the means of each experimental group, with the bar representing one standard error. Significance ($P \leqslant 0.05$) is indicated by an asterisk.

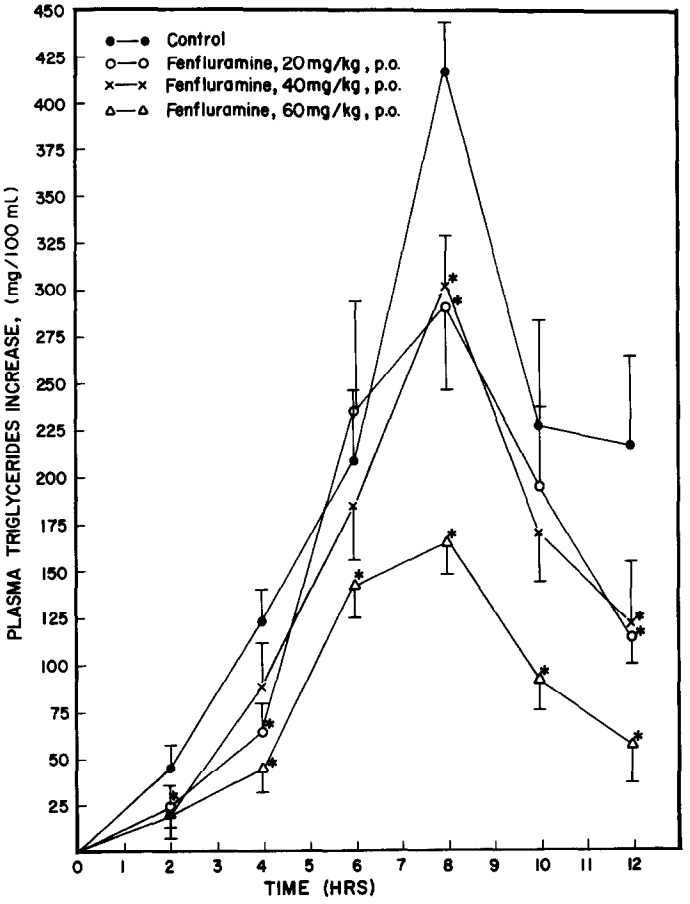

Fig. 2. Triglyceride absorption in fenfluramine-treated rats. Corn oil (20 ml/kg, p.o.) was administered 15 min after the following oral doses of fenfluramine in 1% gum arabic: 20 mg/kg (O-O), 40 mg/kg(X-X) and 60 mg/kg (△-△). Controls received 1% gum arabic (●-●). There were eight rats per experimental group. Blood samples were obtained from the tails at the indicated times in heparinized tubes. Plasmas were assayed for triglycerides [20]. Values are the means of each experimental group. The bar represents one standard error. Significance ($P \leqslant 0.05$) is indicated by an asterisk.

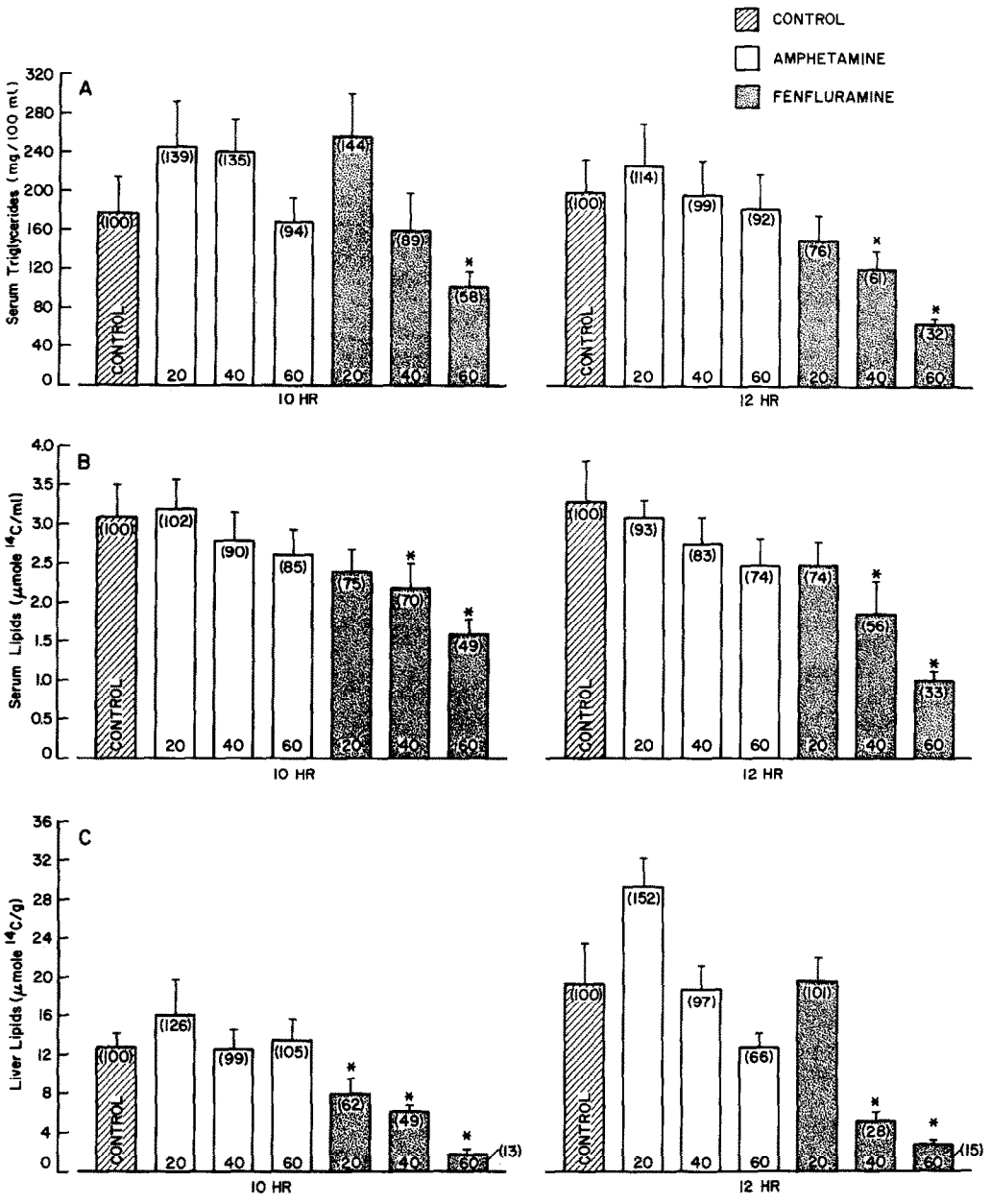

Fig. 3. Serum triglyceride and lipid levels in amphetamine and fenfluramine-treated rats. Rats (eight per group) were administered [^{14}C]-corn oil (20 mg/kg), 15 min after amphetamine (20, 40 and 60 mg/kg, p.o.) or fenfluramine (20, 40 and 60 mg/kg, p.o.) in 1% gum arabic. Controls received 1% gum arabic. Rats were killed 10 and 12 hr later. Serum triglycerides [20] and serum and liver lipids were analyzed [24]. The values are the mean of each experimental group with the bar representing one standard error. Significance ($P \leq 0.05$) is indicated by the asterisk. Each number in parentheses is the per cent of control. The number at the bottom of each column is the dose of amphetamine (unshaded areas) or fenfluramine (shaded areas) in mg/kg.

Serum triglycerides (mg/100 ml) were depressed significantly by fenfluramine in a dose-related manner (Fig. 3A). The decrease was significant at 10 and 12 hr. Amphetamine produced small elevations in serum triglycerides at the 20 and 40 mg/kg doses at 10 hr and at the 20 mg/kg dose at 12 hr.

The radioactivity in the serum followed the trends observed in serum triglycerides (Fig. 3B). There was a small but not significant dose-dependent decrease in serum radioactivity in those rats treated with amphetamine. In rats treated with fenfluramine, there were dose-related decreases in serum radioactivity which were significant at 40 and 60 mg/kg.

A striking difference was observed in the accumulation of radioactive liver lipids in rats treated with either amphetamine or fenfluramine (Fig. 3C). Amphetamine at all doses and both times produced no significant effect on accumulation of liver lipid, compared to controls. On the other hand, fenfluramine at 20, 40 and 60 mg/kg produced significant

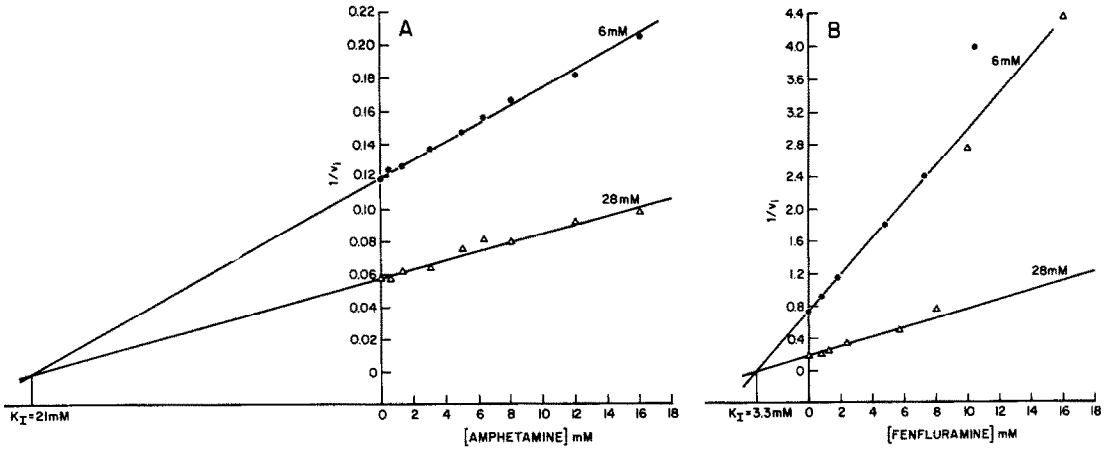

Fig. 4. Dixon plots of amphetamine and fenfluramine inhibition of pancreatic lipase. The indicated amount of inhibitor in 90% ethanol (maximum volume 200 μl) was added to the substrate emulsion and mixed thoroughly. Partially purified pancreatic lipase (10 μg) was used to initiate the reaction. The free fatty acids liberated (v_i = μmoles/min) were continuously titrated using a recording pH Stat. Each point is the average of duplicate assays. Key: panel A, amphetamine; and panel B, fenfluramine.

decreases in liver lipid accumulation at both 10 and 12 hr.

Inhibition of pancreatic lipase by amphetamine and fenfluramine. Inhibition of absorption of corn oil led to a study of the inhibition of pancreatic lipase by amphetamine and fenfluramine (Fig. 4, panels A and B). Initial velocity measurements of the effect of amphetamine on pancreatic lipase revealed amphetamine to be a weak inhibitor, giving a K_i of 21 mM as determined from the Dixon plot (Fig. 4A). In contrast, fenfluramine was a more potent inhibitor, K_i = 3.3 mM (Fig. 4B). The graphical displays emphasize the cooperative inhibitory properties of fenfluramine and the classical competitive inhibitor behavior of amphetamine.

DISCUSSION

The results obtained in the present studies reflect biochemical actions of amphetamine and fenfluramine which are not related to decreased food consumption.

This report demonstrates inhibition of hepatic cholesterogenesis by amphetamine and fenfluramine, both *in vitro* and *in vivo*. Fenfluramine was significantly more potent than amphetamine in the isolated hepatocyte study (Table 1) and slightly more inhibitory in the *in vivo* synthesis experiments (Table 3). The inhibition of cholesterogenesis by fenfluramine may be responsible for the decreased plasma cholesterol levels reported in some studies in patients receiving fenfluramine [32, 33]. The serum cholesterol levels in the present studies were unaffected, which was not surprising since the experiments were of an acute nature.

The data also suggest differences between amphetamine and fenfluramine in their capacity to inhibit fatty acid synthesis. Fenfluramine was approximately four times more potent an inhibitor of fatty acid synthesis in isolated hepatocytes as compared to amphetamine. However, fenfluramine appeared toxic to isolated hepatocytes, as indicated by the inhibition of CO_2 production, and this may have augmented the observed inhibition of fatty acid synthesis. In contrast, amphetamine stimulated CO_2 production in isolated hepatocytes at all concentrations tested (Table 1) and was not an inhibitor of fatty acid synthesis. The same general conclusions were drawn from the *in vivo* fatty acid synthesis experiment (Table 2). Fenfluramine significantly inhibited fatty acid synthesis in liver, intestine and adipose while amphetamine, except at 20 mg/kg in intestine, was not an inhibitor. These results extend the previously reported *in vitro* studies on triglyceride synthesis in intestine and adipose tissue [7, 10–13].

Our data show a dose-related reduction in the absorption of corn oil by fenfluramine (Figs. 2 and 3). However, significant reductions were observed only at the high doses, 40 and 60 mg/kg. These data are in general agreement with those of Garattini *et al.* [34]. In contrast to the data of Garattini *et al.*, we were not able to depress significantly corn oil absorption with amphetamine at doses three times greater than those used in their experiments. We did observe, however, a significant delay in the absorption of corn oil. These discrepancies seem to be related to experimental timing. Maximum lipid absorption in the corn oil-loaded rat model has been shown by us to occur at least 8 hr after corn oil administration (Figs. 1 and 2). Garattini *et al.* [34] observed plasma lipid levels only at 2 hr after lipid administration. Their conclusion that amphetamine inhibited lipid absorption may have been different had their observations taken place nearer the peak of lipid absorption.

The utility of classical enzyme kinetics in studies of complex emulsion systems such as pancreatic lipase is evident in the use of Dixon plots for determining the effectiveness of amphetamine and fenfluramine as inhibitors of pancreatic lipase (Fig. 4). The graphical displays show the weakly competitive nature of amphetamine (K_i = 21 mM) and the strong cooperative inhibitory properties of fenfluramine (K_i = 3.3 mM). The K_i for fenfluramine was determined from concentrations of fenfluramine to 8 mM. Above 8 mM, the inhibition of pancreatic lipase was exponential (Fig. 4B). This cooperative inhibition may be due to

alterations in the surface properties of the emulsified substrate or a direct enzyme–fenfluramine interaction.

A critical analysis of the concentrations at which effects on fatty acid synthesis and lipid absorption were observed indicates that fenfluramine may be more effective *in vivo* than *in vitro*. If body weight were converted to volume, assuming 75% water, then the concentration of fenfluramine, administered in the *in vivo* synthesis experiment, which elicited significant inhibition would be 0.03 mM (i.p.), assuming no inactivating metabolism of fenfluramine occurred. This value is more than 10-fold lower than the concentration required for 50 per cent inhibition of synthesis in the *in vitro* rat isolated hepatocyte experiment (2 mM). The inhibition *in vitro* of lipase by fenfluramine suggested that the reduction in appearance of plasma triglycerides from corn oil might be due to inhibition of intestinal absorption. However, the *in vitro* K_i for fenfluramine inhibition of lipase (3.3 mM) was 10-fold higher than the *in vivo* active dose of 0.2 mM, p.o. (assuming 75 per cent body water as above). Of course, the concentration of fenfluramine in the lumen of the small intestine might be considerably higher than 0.2 mM. The effects of fenfluramine on lipid absorption might have been due also to inhibition of phosphatidate phosphohydrolase, as previously reported [10].

In conclusion, this study emphasizes the importance of evaluating potential peripheral metabolic effects in the pharmacological analyses of antiobesity compounds. Considering that the therapeutic dose in man is approximately 1 to 2 mg/kg, the peripheral metabolic effects reported here are probably not an important aspect of the antiobesity action of fenfluramine.

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