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Studies on the ethanol-induced changes in glycerolipid synthesis in rats and their partial reversal by *N*-(2-benzoyloxyethyl)norfenfluramine (benfluorex)

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Rats were treated daily for 5 days with benfluorex (S-780) 50 mg kg^{-1} and 2 h after the fifth treatment were intubated with ethanol 5 g kg^{-1} or with isocaloric glucose. S-780 did not alter the absorption or oxidation of [1-^{14}C]ethanol nor did it decrease the concentration of ethanol and its water soluble metabolites in the tissues examined. The rate of hepatic glycerolipid synthesis was measured *in vivo* using [^{14}C]palmitate and [^{3}H]glycerol and the accumulation of [^{14}C]palmitate in various tissues was determined 4 h after intravenous injection. Ethanol treatment increased the relative rate of TG† synthesis in the liver and decreased the synthesis of PC, PE and PS + PI. The relative flux from PA to DG and TG was increased. These changes were reflected in the accumulation of [^{14}C]palmitate in hepatic lipids and were obtained under conditions where the net rate of [^{14}C]palmitate oxidation to $^{14}CO_2$ was greater in those rats treated with ethanol instead of glucose. S-780 did not alter the oxidation rates over a 4 h period. Ethanol treatment increased the accumulation of [^{14}C]palmitate in the PS + PI fraction of lung and decreased the proportion in PE. In kidney it increased the concentration of [^{14}C]palmitate in TG, DG and PE and decreased the concentration in PC and PS + PI. Pretreatment of the rats with S-780 partially reversed the changes in palmitate accumulation in glycerolipids which were caused by ethanol. S-780 treatment also decreased the rate of hepatic TG synthesis from glycerol in the rats fed glucose. It increased the percentage recovery of [^{14}C]palmitate in the livers and kidneys of glucose fed rats and decreased that in adipose tissue. This treatment decreased the proportion of [^{14}C]palmitate recovered in the TG of the kidneys and lungs of these rats and increased the proportion in PI + PS. The results are discussed in relation to the mechanisms by which ethanol and S-780 are thought to interfere with glycerolipid synthesis.

It is well established that ingestion of ethanol stimulates the incorporation of long chain fatty acids into TG† in the liver (Lieber, 1974). This gives rise to a fatty liver and if continued produces a hypertriglyceridaemia. Conversely, it is known that fenfluramine and its derivatives such as *N*-(2-benzoyloxyethyl)norfenfluramine (benfluorex or S-780) decreases the rates of TG synthesis in a number of tissues (Wilson & Galton, 1971; Marsh & Bizzi, 1972; Dannenburg, Kardian & Norrell, 1973; Brindley & Bowley, 1975). Treatment of rats with S-780 reduces the concentration of hepatic TG (Duhault, Boulanger & others, 1976) and also the concentration of serum TG (Riveline, 1975; Kaye, Tomlin & Galton, 1975; Duhault & others, 1976). It was therefore thought that S-780 might reverse the ethanol-induced synthesis of TG. The present experiments were designed to test this hypothesis and to gain information about the effects of ethanol and S-780 on lipid metabolism.

In vitro amphiphilic cationic drugs such as the derivatives of fenfluramine inhibit PA phosphohydrolase (EC 3.1.3.4) (Brindley & Bowley, 1975) and stimulate PA cytidylyltransferase (EC 2.7.7.41) (Sturton & Brindley, 1977). This leads to the redirection of lipid synthesis away from the formation of TG and the zwitterionic lipids (PC and PE) and towards the production of acidic glycerolipids (Brindley, Allan & Michell, 1975; Michell, Allan & others, 1976; Brindley, Bowley & others, 1977). Experiments performed after the chronic treatment of rats with S-780 showed that this drug decreases the hepatic synthesis *in vivo* of PC and PE. The synthesis of TG in liver slices obtained from these rats was also decreased (Brindley, Bowley & others, 1976). However, there was little indication that S-780 increased the synthesis of acidic phospholipids *in vivo* and no significant decrease in the conversion of PA to DG was observed. It was hoped that rats exhibiting an ethanol-induced synthesis of TG might provide a suitable experimental model for investigating further

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† Abbreviations: PS = phosphatidylserine, PI = phosphatidylinositol, PE = phosphatidylethanolamine, PC = phosphatidylcholine, PA = phosphatidate, DG = diacylglycerol, TG = triacylglycerol.

the mechanisms by which S-780 decreases the synthesis of TG *in vivo*.

MATERIALS AND METHODS

Materials

The source of most of the materials has already been described (Brindley & others, 1976). β-Phenethylamine was obtained from BDH Chemicals Ltd, Poole, Dorset, U.K. and Nuclear Chicago Solubilizer (NCS) was obtained through Hopkin and Williams, Chadwell Heath, Essex, U.K. Radioactive compounds were purchased from the Radiochemical Centre, Amersham, Bucks., U.K.

Treatment of rats

Male Wistar rats (approx. 200 g) were treated daily for 5 days with S-780 50 mg kg^{-1} (Brindley & others, 1976). Control rats received the equivalent volume of 0·5% (w/v) gum tragacanth suspension and all rats were allowed free access to the 41B diet and water. Two h after treatment on the fifth day the rats were intubated with ethanol 5g kg^{-1}, in a 20% (v/v) solution, or with isocaloric glucose. They were then deprived of food but not of water.

Measurement of the effect of S-780 on the metabolism of ethanol

Rats were treated as above except that the ethanol contained 10 μCi [1-^{14}C]ethanol. Each rat was then placed in a sealed Jencons Metabowl MkIII metabolic cage. Air was drawn through the apparatus at 250–350 ml min^{-1} and bubbled through three traps in series each holding 20 ml of ice-cold β-phenethylamine–methanol–xylene (27:27:46; by vol) containing 2,5-diphenyloxazole, 4 g $litre^{-1}$, and 1,4 bis-(5-diphenyloxazol-2-yl) benzene, 0·1g $litre^{-1}$ (Woeller, 1961). At hourly intervals, two samples (1 ml) were removed from each trap and radioactivity was determined by adding 10 ml of methanol-xylene scintillator (Opperman, Nystrom & others, 1959) and counting in a Packard 3255 TriCarb Spectrometer. The total contents of the first trap were replaced every hour. Traps 1, 2 and 3 trapped an average of 97, 3 and 0% of the $^{14}CO_2$ produced respectively. Recovery was 100% s.d. 3% (n = 4 experiments) as determined by releasing a known quantity of $^{14}CO_2$ from sodium [^{14}C]bicarbonate with HCl.

The expired CO_2 from the rats was measured for 6 h, after which the animals were killed by cervical dislocation. Samples of tissue (approx. 1 g) were quickly excised, frozen in liquid nitrogen and placed in cooled weighed tubes. Lipids were extracted from these samples and the radioactivity in the lipid and aqueous phases determined (Brindley & others, 1976). The whole gastrointestinal tract was washed out with ice-cold 0·15 M NaCl. These washings were macerated in a chilled blender, centrifuged to remove debris, and adjusted to 250 ml. Samples were assayed for radioactivity (Brindley & others, 1976) to estimate the unabsorbed ethanol.

Measurement of the effects of ethanol and S-780 on the metabolism of injected potassium [U-^{14}C]palmitate in vivo

Rats were treated with S-780 or gum tragacanth followed by ethanol or glucose as described above. After 5 h the rats were lightly anaesthetized with diethyl ether and their tails warmed in water. An injection into the tail vein of potassium-[U-^{14}C]-palmitate (256 μCi μmol^{-1} in 0·5 ml) 97·5 nmol kg^{-1} was made. The palmitate was bound to the albumin of rat serum as described by Borgström & Olivecrona (1961). The rats were then placed into individual metabolic cages and the production of $^{14}CO_2$ was measured for 4 h as described above. The rats were then killed and samples were quickly excised and frozen. Approximately 100 mg duplicate samples, either as whole tissues or 1/3 (v/v) homogenates in 0·15 M NaCl, were solubilized using 1 ml NCS solubilizer. Digestion, decolourization and scintillation counting were performed according to the manufacturer's instructions. Lipids were extracted and analysed as described by Brindley & others (1976).

Measurement of the effect of S-780 and ethanol on hepatic glycerolipid synthesis in vivo

Six hours after ethanol feeding the rate of glycerolipid synthesis in the liver was measured 1 min after the intraportal injection of potassium [^{14}C]palmitate and [1,3-^{3}H]glycerol as previously described (Brindley & others, 1976).

RESULTS

The effects of S-780 on the absorption, distribution and metabolism of ethanol

S-780-treatment did not alter the percentage absorption of [^{14}C]ethanol which averaged 85% after 6 h. At this time about 19% of the dose was expired as CO_2 and the rate of ethanol oxidation and its excretion through the lungs and in urine was not significantly influenced by S-780 administration. There was also no difference in the percentage of the absorbed [^{14}C]ethanol recovered in the liver, heart and adipose tissue. The percentage of absorbed [^{14}C]ethanol g^{-1} of tissue in the blood, lungs and kidneys of 7 control

rats was 0·14 (s.d. 0·05), 0·14 (s.d. 0·05) and 0·18 (s.d. 0·06)% respectively. The equivalent values were significantly greater ($P<0·05$) in eight S-780-treated rats and these were respectively 0·21 s.d. 0·07, 0·21 s.d. 0·06 and 0·26 s.d. 0·07%. There were no significant differences in the percentage incorporation of [^{14}C]ethanol in the tissue lipids of the control and S-780 treated rats. The distribution of ^{14}C from ethanol in the tissues was similar to that discussed by Kalant (1971).

The effect of S-780 and ethanol on the metabolism of [U-^{14}C]palmitate injected intravenously into rats

The effects of S-780 and ethanol on the metabolism and tissue distribution of intravenously injected [^{14}C]palmitate was measured over 4 h (Table 1). The rate of production of $^{14}CO_2$ was significantly greater in the rats treated with ethanol than in those treated with isocaloric glucose. Pretreatment of the rats with S-780 had no significant effect on the percentage of the injected palmitate which was oxidized to CO_2 in the rats given either ethanol or glucose. The quantity of ^{14}C excreted in the urine was higher in the rats treated with ethanol than in those given glucose. S-780 treatment decreased the recovery of ^{14}C in the urine of rats given glucose, but not of those given ethanol.

Ethanol has been shown to inhibit β-oxidation in liver slices (Lieber & Schmid, 1961) and perfused liver (Lieber, Lefèvre & others, 1967) and is thought to be part of the mechanism causing the ethanol-induced fatty liver (Lieber, 1974). Wooles & Weymouth (1968) showed that ethanol reduced CO_2 production from fatty acids by rats but the controls in their experiments were treated with saline rather than isocaloric glucose. Replacement of the ethanol with glucose produces a further reduction in CO_2 production (Rebouças & Isselbacher, 1961) and the results in Table 1 are in agreement with this. Although ethanol can inhibit hepatic β-oxidation it may produce increases or very little change in the rate of ketone body production (Rawat, 1968; Wotjtczat, 1968; Williamson, Scholz & others, 1969; Fellinius & Kiessling, 1973). These ketones can then be oxidized by extrahepatic tissues. The present experiments reflect the net rate of [^{14}C]palmitate oxidation in all tissues.

Control rats given ethanol had lower concentrations of ^{14}C in the lungs and heart than did the controls given glucose (Table 1). Pretreatment with S-780 before intubation with ethanol decreased the ^{14}C concentration in heart and kidneys. S-780 treated rats given ethanol had less ^{14}C in lungs, heart and kidneys than did the S-780 treated rats given glucose. The latter rats had more ^{14}C g^{-1} of liver and kidneys, but less in adipose tissue than did the control rats given glucose.

Control rats given ethanol incorporated relatively more [^{14}C]palmitate into liver TG, and relatively less into PC, PE and PI + PS than did control rats given glucose. Pretreatment with S-780 reduced the ethanol-induced incorporation into TG and increased the incorporation into PC. However, S-780 did not completely reverse the effects of ethanol in altering the pattern of incorporation of [^{14}C]palmitate into lipids (Groups III *vs* IV, Table 2), neither did it alter the [^{14}C] labelling pattern of hepatic lipids in rats fed glucose (Groups I *vs* IV, Table 2).

In the lungs ethanol did not change the proportion of [^{14}C]palmitate in the TG of the control rats (see Table 2). It did, however, increase the relative concentration of ^{14}C in PI + PS and decrease the

Table 1. *The effects of S-780 and ethanol on the oxidation and tissue distribution of [^{14}C]palmitate in the rat.* Rats were treated daily for 5 days with S-780 (50 mg kg^{-1}) or with gum tragacanth (controls). Two h after the fifth intubation they were given by stomach tube either ethanol (5 g kg^{-1}) or isocaloric glucose. After a further 5 h they were injected with [^{14}C]palmitate (Materials and Methods section). Results are expressed as means (with 1 s.d.) (n = no of rats). The significance of differences between the groups is indicated by $*P<0·05$; $**P<0·02$; $***P<0·01$; $\dagger P<0·005$; $\dagger\dagger P<0·001$.

Total ^{14}C in	Time‡ (h)	% of [^{14}C] recovered			
		(I) Cont. + gluc. (n = 8)	(II) Cont. + eth. (n = 9)	(III) S-780 + eth. (n = 11)	(IV) S-780 + gluc. (n = 8)
Expired CO_2	1	5(3)	8(3)	9(2) III *vs* IV††	3(2)
	2	11(4) I *vs* II*	16(4)	17(3) III *vs* IV††	9(4)
	3	16(5) I *vs* II*	22(4)	22(3) III *vs* IV††	14(4)
	4	21(5) I *vs* II*	26(4)	27(4) III *vs* IV††	19(5)
Urine	4	0·31(0·06) I *vs* II*	0·49(0·18)	0·54(0·16) III *vs* IV**	0·23(0·02) I *vs* IV**
		% of ^{14}C recovered g^{-1} of tissue			
Liver	4	1·8(0·2)	2·2(0·5)	2·6(0·5)	2·3(0·4) I *vs* IV**
Blood	4	0·08(0·01)	0·08(0·01)	0·09(0·02)	0·08(0·01)
Lung	4	1·3(0·2) I *vs* II*	1·1(0·3)	1·2(0·2) III *vs* IV**	1·5(0·3)
Adipose tissue	4	0·29(0·12)	0·19(0·10)	0·17(0·11)	0·16(0·04) I *vs* IV**
Heart	4	0·51(0·12) I *vs* II***	0·35(0·09) II *vs* III**	0·40(0·06) III *vs* IV†	0·67(0·25)
Kidney	4	1·0(0·1)	0·95(0·1) II *vs* III†	1·1(0·07) III *vs* IV**	1·2(0·1) I *vs* IV**

‡ Time after injection with [^{14}C]palmitate.

Table 2. *The effects of S-780 and ethanol on the incorporation of [^{14}C]palmitate into various lipids of rat liver, lung and kidney.* The distribution of ^{14}C in the lipids obtained from the rats described in Table 1 is shown as means percentages (with l.s.d.). The percentages of the total ^{14}C isolated from liver, lung and kidneys were 98 ± 1, 97 ± 1 and 95 ± 2 % respectively. The significance of the differences is shown as described in Table 1 (n = no. of rats).

			% [^{14}C]palmitate incorporated into glycerolipid:				
Tissue		Group	PS + PI	PC	PE	DG	TG
Liver	(I)	Control + glucose (n = 8)	4(1) I *vs* II†	59(2) I *vs* II††	22(1) I *vs* II†	5(0·5)	8(2) I *vs* II††
	(II)	Control + ethanol (n = 8)	3(0·9)	42(6) II *vs* III***	18(3)	4(2)	31(10) II *vs* III*
	(III)	S-780 treated + ethanol (n = 10)	3(1) III *vs* IV*	50(7) III *vs* IV***	20(2) III *vs* IV**	4(1)	20(11) III *vs* IV***
	(IV)	S-780 treated + glucose (n = 8)	5(2)	59(3)	23(2)	4(0·5)	7(4)
Lung	(I)	Control + glucose (n = 7)	4(1) I *vs* II**	64(7)	7(1) I *vs* II*	6(1)	18(9)
	(II)	Control + ethanol (n = 9)	6(2)	61(9)	5(2)	7(3) II *vs* III*	17(7)
	(III)	S-780 treated + ethanol (n = 11)	7(3)	67(5)	6(1) III *vs* IV††	5(0·6) III *vs* IV††	13(5) III *vs* IV**
	(IV)	S-780 treated + glucose (n = 8)	6(0·7) I *vs* IV††	69(4)	8(1)	6(1)	8(3) I *vs* IV**
Kidney	(I)	Control + glucose (n = 8)	7(1) I *vs* II*	62(4) I *vs* II††	10(0·9) I *vs* II†	5(0·6) I *vs* II*	14(5) I *vs* II†
	(II)	Control + ethanol (n = 9)	6(0·6) II *vs* III†	47(3) II *vs* III††	14(3)	6(2) II *vs* III***	23(6) II *vs* III†
	(III)	S-780 treated + ethanol (n = 11)	7(0·9) III *vs* IV†	53(3) III *vs* IV††	16(4) III *vs* IV†	4(1)	17(4) III *vs* IV††
	(IV)	S-780 treated + glucose (n = 7)	9(1) I *vs* IV†	64(2)	11(0·6)	5(0·5)	9(3) I *vs* IV*

proportion in PE. In S-780-treated rats, ethanol increased the proportion of the ^{14}C in TG and decreased the proportions in DG and PE. Treatment with S-780 did not cause a significant decrease in the proportion of ^{14}C in TG in the rats given ethanol, but did have this effect in the rats fed glucose. The latter change was accompanied by an increase in the proportion of ^{14}C in the acidic lipids, PI + PS.

The effects of ethanol in the kidneys of the control rats were to increase the proportion of [^{14}C]palmitate in TG, DG and PE and to decrease the proportion in PC and in PS + PI (Table 3). Pretreatment of the rats with S-780 partially prevented these changes. S-780 decreased the proportion of the ^{14}C present in TG of the rats given glucose and increased it in the PS + PI fraction.

The effects of S-780 and ethanol on the synthesis of hepatic glycerolipids

These experiments were performed to assess whether alterations in the rates of glycerolipid synthesis could explain the results described in Tables 1 and 2. Rats were treated with either 5 g (20% v/v solution) or 7·5 g (30% v/v solution) of ethanol kg^{-1} and control rats were given isocaloric glucose. The effects of ethanol on hepatic glycerolipid synthesis at these two dose levels were similar, but were more marked at the lower dose. This was probably caused by an increased absorption of ethanol at the lower dose (Benson, 1973). The results in Tables 3 and 4 are expressed both in terms of the d min^{-1} of isotope incorporated and as the relative distribution of label in the different lipid fractions for the reasons described earlier (Brindley & others, 1976).

(A) *Control rats treated with glucose compared with control rats treated with ethanol*

Ethanol treatment increased the absolute incorporation of [^{3}H]glycerol and [^{14}C]palmitate into TG and decreased the accumulation of [^{14}C]palmitate in PA and in PI + PS (Tables 3 and 4). The incorporation of these precursors into total glycerolipids was higher in the ethanol treated rats, but this increase was only significant for [^{3}H]glycerol. This is probably caused by the ethanol-induced increase in hepatic

Table 3. *The effects of ethanol and S-780 on the synthesis of glycerolipids from* [^{14}C]*palmitate by rat liver* in vivo. Rats were treated daily with S-780 or gum tragacanth suspension (controls) for 5 days and then given either ethanol or isocaloric glucose (Materials and Methods section). Lipid synthesis was measured 1 min after intraportal injection of [^{14}C]palmitate and [^{3}H]glycerol. The results are expressed as means (with ls.d.) (n = no. of rats). Significant differences in the groups are indicated as described in Table 1.

	Incorporation of [^{14}C]palmitate							
	dmin^{-1} × 10^{-3} min^{-1} per liver				dmin^{-1}/100 dmin^{-1}			
	(I) control + glucose (n = 9)	(II) control + ethanol (n = 11)	(III) S-780 + ethanol (n = 11)	(IV) S-780 + glucose (n = 10)	(I) control + glucose (n = 9)	(II) control + ethanol (n = 11)	(III) S-780 + ethanol (n = 11)	(IV) S-780 + glucose (n = 11)
PS + PI	68(46)	39(18)	41(22)	60(31)	3(2)	1·5(0·7)	2(1)	3(1)
	I *vs* II*				I *vs* II***			
PC	336(90)	354(209)	291(82)	408(134)	17(2)	14(2)	16(3)	16(2)
			III *vs* IV*		I *vs* II***			
PE	109(32)	97(53)	82(28)	127(46)	5(0·5)	4(1)	4(1)	5(1)
			III *vs* IV**		I *vs* II††		III *vs* IV*	
PA	443(119)	256(193)	276(121)	518(69)	21(3)	10(3)	15(5)	23(5)
	I *vs* II*		III *vs* IV††		I *vs* II††	II *vs* III***	III *vs* IV†	
DG	626(139)	529(312)	556(162)	810(265)	31(3)	21(3)	30(2)	33(3)
			III *vs* IV**		I *vs* II††	II *vs* III††	III *vs* IV*	
TG	443(111)	1129(475)	621(467)	509(219)	22(3)	48(7)	32(9)	20(4)
	I *vs* II††	II *vs* III**			I *vs* II††	II *vs* III††	III *vs* IV†††	
Flux from PA → DG	1516(340)	2104(955)	1550(547)	1853(619)	75(4)	88(3)	82(5)	74(5)
					I *vs* II††	II *vs* III†	III *vs* IV†	
Total lipid	2031(487)	2425(1152)	1867(610)	2431(737)	—	—	—	—
Total ^{14}C	2900(574)	3729(1355)	2802(727)	3547(810)	—	—	—	—
			III *vs* IV*					

redox state and the corresponding accumulation of [^{3}H]glycerol in glycerol phosphate (Fellinius, Bengtsson & Kiessling, 1973). Incorporation of ^{3}H into lipid from glycerol phosphate would therefore have been favoured against the conversion to glucose and lactate.

Ethanol treatment increased the percentage of [^{3}H]glycerol and [^{14}C]palmitate recovered in TG and the flux of these precursors from PA to DG. The proportion of these precursors in other lipids was lowered in the ethanol-treated rats.

(B) *Comparison of the control and S-780 groups treated with ethanol*

S-780 decreased the incorporation (in d min^{-1}) of both [^{14}C]palmitate and [^{3}H]glycerol into TG (Tables 3 and 4). It also increased the accumulation of [^{3}H]glycerol in the other lipid classes. The percentage recovery of both precursors in TG was decreased by treatment with S-780, whereas the relative accumulation in DG and in PA was increased. The relative incorporation of [^{3}H]glycerol into PI + PS was also increased by S-780 treatment. S-780 therefore tended to reverse the effects of ethanol and decreased the flux of substrates from PA to DG.

(C) *Comparison of the S-780-treated rats given ethanol and glucose*

Ethanol increased the incorporation of [^{3}H]glycerol into lipids particularly into TG (Table 4). By contrast there was a reduction (though not significant) in the mean incorporation of [^{14}C]palmitate into lipids which was associated with a significant decrease in the accumulation of ^{14}C in DG, PA, PE and PC (Table 3). However, when considered in terms of the percentage of radioactivity in different lipids classes the effects of ethanol on the incorporation of [^{14}C]-palmitate and [^{3}H]glycerol were similar. Ethanol increased the flux of these precursors from PA to DG and stimulated the synthesis of TG. It decreased the relative accumulation of ^{14}C in DG, PA and PC (Table 3) and that of [^{3}H]glycerol in PA and PC (Table 4).

(D) *Comparison of the control and S-780 treated rats given glucose*

The only significant differences were found in the relative distribution of [^{3}H]glycerol (Table 4). Treatment with S-780 decreased the relative rate of TG synthesis and increased the appearance of radioactivity in DG.

Table 4. *The effects of ethanol and S-780 on the synthesis of glycerolipids from [^{3}H]glycerol by rat liver* in vivo. Glycerolipid synthesis from [^{3}H]glycerol was also measured using the rats described in Table 3 and the results are expressed in the same way.

	Incorporation of [^{3}H]glycerol							
	d min^{-1} × 10^{-3} min^{-1} per liver				d min^{-1}/100 d min^{-1}			
	(I) control + glucose (n = 9)	(II) control + ethanol (n = 11)	(III) S-780 + ethanol (n = 11)	(IV) S-780 + glucose (n = 10)	(I) control + glucose (n = 9)	(II) control + ethanol (n = 11)	(III) S-780 + ethanol (n = 11)	(IV) S-780 + glucose (n = 11)
PS + PI	140(120)	104(60)	180(80)	124(108)	2(2)	1(0·6)	2(1)	2(1)
		II *vs* III*			I *vs* II*	II *vs* III*		
PC	639(228)	647(284)	899(276)	647(216)	11(2)	7(2)	9(2)	11(1)
		II *vs* III*	III *vs* IV*		I *vs* II††	II *vs* III*	III *vs* IV*	
PE	288(144)	316(144)	452(144)	300(96)	5(1)	4(1·5)	5(1)	5(1)
		II *vs* III*	III *vs* IV*		I *vs* II*			
PA	1283(436)	935(779)	1734(947)	1411(364)	22(5)	10(5)	17(7)	24(4)
		II *vs* III*			I *vs* II††	II *vs* III***	III *vs* IV***	
DG	1894(567)	2058(1123)	3540(1035)	2338(715)	34(5)	22(6)	35(2)	38(5)
		II *vs* III†	III *vs* IV**		I *vs* II††	II *vs* III††		I *vs* IV*
TG	1423(607)	4623(947)	3093(1379)	1099(324)	25(6)	57(16)	31(8)	18(5)
	I *vs* II††	II *vs* III**	III *vs* IV††		I *vs* II††	II *vs* III††	III *vs* IV††	I *vs* IV*
Flux from PA → DG	3864(1910)	7644(2106)	7984(2450)	4204(779)	74(6)	88(5)	80(7)	72(3)
			III *vs* IV††		I *vs* II††	II *vs* III***	III *vs* IV*	
Total lipid	5954(2006)	9375(2585)	9914(2893)	5974(1463)	—	—	—	—
	I *vs* II†		III *vs* IV†					
Non-lipid ^{3}H	49662(11217)	43029(16563)	52991(17337)	57982(13007)	—	—	—	—

DISCUSSION

S-780 treatment did not affect the absorption of ethanol by rats, its rate of oxidation, nor did it decrease the concentration of ethanol and its water-soluble metabolites in the tissues investigated. It is therefore concluded that S-780 interferes directly with the effects of ethanol on lipid metabolism. Wooles & Weymouth (1968) reversed the ethanol-induced fatty liver by pretreating rats with chlorcyclizine. They claimed that this resulted from a chlorcyclizine-induced increase in β-oxidation and a decrease in TG synthesis. However, they used saline-treated controls rather than the glucose-treated controls employed in the present work. Chlorcyclizine, like S-780 and some of its metabolites, is an amphiphilic amine and so could have similar effects on lipid metabolism (Brindley & others, 1975). The reversal of the ethanol-induced TG synthesis in the present experiments was not accompanied by a net increase in the rate of β-oxidation. However, a stimulation of β-oxidation by S-780 may have occurred in some tissues. Feeding glucose rather than ethanol depressed the net rate of β-oxidation (Table 1), but the rate of hepatic TG synthesis was higher in the ethanol treated rats (Tables 3 and 4). Ethanol therefore appears to have a direct action in stimulating the synthesis of TG. Similar conclusions were made by Abrams & Cooper (1976). It must however, be stressed that the present experiments measured the relative rates of metabolism of exogenously administered substrates and do not take account of dilution from endogenous sources.

The enzymic mechanism by which ethanol interferes with glycerolipid synthesis is not known. The results in Tables 3 and 4 show that ethanol increases the flux of metabolites from PA to DG and to TG. This is accompanied by a decrease in the relative rate of synthesis of PC, PE and PS + PI. Other work has demonstrated that ingestion of ethanol and isopropanol increases the rate of hepatic TG synthesis at the expense of phospholipid production (Scheig & Isselbacher, 1965; Beaugé, Clément & others, 1972; Abrams & Cooper, 1976). These authors and Estler (1974) suggested that the stimulation of TG synthesis and the steatosis result from a direct effect on glycerolipid synthesis rather than from changes in hepatic redox state.

S-780 partially reverses these ethanol-induced effects on glycerolipid synthesis. In particular it decreases the flux from PA to DG and increases the accumulation of [^{14}C]palmitate and [^{3}H]glycerol in PA (Tables 3 and 4). This observation is compatible with the hypothesis that an inhibition of PA phosphohydrolase is involved in the S-780-induced decrease in TG synthesis (Brindley & others, 1975; Sturton & Brindley, 1977). The inhibition of TG synthesis by S-780 was greater in the ethanol-treated rats than in those given glucose (Tables 3 and 4). It was not observed *in vivo* in experiments where the rats were not intubated with glucose or ethanol (Brindley & others, 1976).

Changes in the rates of glycerolipid synthesis were probably partly responsible for the alteration in the fate of intravenously injected [^{14}C]palmitate (Tables

1 and 2). Ethanol treatment increased the percentage recovery of [^{14}C]palmitate in the TG of kidney and liver. Pretreatment with S-780 decreased the recovery of ^{14}C in adipose tissue of the glucose fed rats (Table 1). It also lowered the concentration of [^{14}C]TG in the lungs and kidneys of glucose-fed rats and that in the liver and kidneys of the ethanol treated rats (Table 2). The S-780-induced increase in the concentration of ^{14}C in the PS + PI fractions of the kidneys and lungs of glucose-fed rats is suggestive of a redirection of glycerolipid synthesis at the level of PA. This is also supported by the S-780-stimulated increase in PS + PI production observed in the ethanol-treated rats in Table 4.

The present work shows that S-780 interferes with the balance between phospholipid and TG synthesis *in vivo*. It produces a decrease in the synthesis and accumulation of TG in rats treated both with ethanol and glucose. These properties probably contribute to the therapeutic action of fenfluramine and S-780 in treating obesity and hyperlipidaemia respectively.

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