insignificant effect on sleeping time was observed.

Thus, strategic supplementation of a ration with essential amino acids to improve its biological value offers promise in reducing the toxicity of ethanol and selected barbiturates as well as in mitigating the CNS effects associated with such compounds.

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Metabolic Effects of a New Hypolipidemic Agent, Ciprofibrate

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Abstract □ Ciprofibrate, a new orally effective hypolipidemic agent like clofibrate, suppressed tyloxapol-induced hypercholesterolemia in rats. Ciprofibrate at 10 mg/kg was effective. Clofibrate required a dosage of 180 mg/kg to suppress the tyloxapol effect. Norepinephrine-induced free fatty acid release was inhibited by clofibrate in rats in accordance with earlier findings. Ciprofibrate and lifibrate differed from clofibrate in that, at hypocholesterolemically effective doses, neither inhibited the hormone-sensitive lipase *in vivo*.

Keyphrases □ Ciprofibrate—hypolipidemic effects, effect on hormone-sensitive lipase, compared to clofibrate and lifibrate, rats □ Anticholesterolemic agents—ciprofibrate, effect on serum lipid levels, hormone-sensitive lipase, compared to clofibrate and lifibrate, rats

Evidence that hyperlipidemia is a major risk factor in coronary artery disease (1) has directed interest toward agents that can correct the lipid abnormality. One such agent is clofibrate, 2-(4-chlorophenoxy)-2-methylpropanoic acid ethyl ester, which serves as a model reference. Related agents are being sought that are more effective against hypercholesterolemia as well as against all types of hyperlipoproteinemia.

A previous report (2) presented evidence that ciprofibrate, 2-[4-(2,2-dichlorocyclopropyl)phenoxy]-2-methylpropanoic acid, is at least 100-fold as hypolipidemic in butter fat, cholesterol-fed hyperlipidemic rats as is clofibrate. In this discussion, additional comparisons between ciprofibrate and clofibrate are presented.

EXPERIMENTAL

Tyloxapol[1]-Induced Hypercholesterolemia—The tyloxapol treatment procedure followed that of Garattini *et al.* (3) and of Kariya *et al.* (4). Young adult Sprague–Dawley rats[2], averaging ~300 g each, were maintained on laboratory feed *ad libitum*. Based on preliminary trials, the test agents were given by intubation at one of the following dosages: ciprofibrate at 10 or 22.5 mg/kg/day or clofibrate at 90 or 180 mg/kg/day, each for 4 days. On the 4th day, the rats were each given tyloxapol at 200 mg/kg iv. Anticholesterolemic effects were evaluated on the basis of cholesterol levels in blood samples taken by cardiac puncture 20 hr later.

For analysis, the blood samples were centrifuged, and the serum was separated. Serum cholesterol was estimated colorimetrically according to the Liebermann–Burchard procedure, using a *p*-toluenesulfonic acid catalyst after Turner and Eales (5).

Inhibition of Hormone-Sensitive Lipase—Two studies were conducted in which the hypolipidemic agents were evaulated for their ability to inhibit the hormone-sensitive lipase. In one, clofibrate was compared with ciprofibrate. In the other, clofibrate was compared with lifibrate, bis(4-chlorophenoxy)acetic acid 1-methyl-4-piperidinyl ester (6).

Young adult Sprague–Dawley strain rats[2], averaging ~250–300 g each, were allocated into groups of nine. They were given one test agent at the following dosages: clofibrate at 150 mg/kg, ciprofibrate at 15 mg/kg, or lifibrate at 40 mg/kg. The test agents were given by intubation in a 1% aqueous gum tragacanth suspension (10 ml/kg) before *l*-norepinephrine (as the bitartrate). The vasoconstrictor effects of norepinephrine were inhibited with 100 mg of phenoxybenzamine/kg sc, 1 hr before norepinephrine was given.

Table I—Ciprofibrate and Clofibrate Effectiveness in Protecting Rats against Tyloxapol Hypercholesterolemia (Means ± *SE*)

Test Compound	Intragastric Dosage, mg/kg/day × 4	Tyloxapol[a]	*n*	Serum Cholesterol[b], mg/100 ml
None	None	−	12	75.2 ± 3.2
None	None	+	10	198.7 ± 16.3
Ciprofibrate	10	+	8	80.9 ± 7.9
	22.5		8	73.5 ± 5.7
Clofibrate	90	+	8	132.4 ± 23.7
	180		7	69.7 ± 4.4

[a] At 200 mg/kg iv on the 4th day of medication. [b] Blood taken 20 hr after tyloxapol.

[1] Triton WR-1339 or Triton A-20.
[2] Charles River.

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Table II—Comparison of Clofibrate, Ciprofibrate, and Lifibrate in Suppressing the *l*-Norepinephrine-Induced Increase in Serum Nonesterified Fatty Acids (NEFA) in the Unfasted Rat (Nine Rats per Group, Means ± *SE*)

	Trial 1						Trial 2					
	Control		Clofibrate[a]		Ciprofibrate[a]		Control		Clofibrate		Lifibrate[a]	
l-Norepinephrine Dosage, μg/kg ip	Serum NEFA, μEq/liter	Serum Cholesterol, mg/100 ml	Serum NEFA, μEq/liter	Serum Cholesterol, mg/100 ml	Serum NEFA, μEq/liter	Serum Cholesterol, mg/100 ml	Serum NEFA, μEq/liter	Serum Cholesterol, mg/100 ml	Serum NEFA, μEq/liter	Serum Cholesterol, mg/100 ml	Serum NEFA, μEq/liter	Serum Cholesterol, mg/100 ml
None	152 ± 23	70 ± 7					165 ± 31	60 ± 4				
10	185 ± 19	66 ± 5	134 ± 24	36 ± 2	180 ± 17	42 ± 2	216 ± 59	65 ± 4	215 ± 27	39 ± 2	211 ± 41	44 ± 3
30	351 ± 54	64 ± 2	164 ± 37	35 ± 1	349 ± 30	40 ± 2	332 ± 60	68 ± 3	217 ± 50	38 ± 2	324 ± 47	40 ± 2

[a] Test agent dosage: clofibrate, 150 mg/kg; ciprofibrate, 15 mg/kg; and lifibrate, 40 mg/kg; each given by intubation at 28, 20, and 2 hr before *l*-norepinephrine administration.

The rats were sedated with 55 mg of pentobarbital/kg sc 0.5 hr before norepinephrine. Norepinephrine was given at either 10 or 30 μg/kg ip 10 min before blood was taken by cardiac puncture. The blood was allowed to clot and was centrifuged, and the serum nonesterified fatty acids were estimated by the copper-soap procedure of Duncombe (7). The fatty acids were extracted with chloroform reacted with a copper–triethanolamine reagent, and color was developed with diethyldithiocarbamate for colorimetric estimation.

RESULTS

Tyloxapol-Treated Rats—In accordance with previous findings (3, 4), tyloxapol at 200 mg/kg doubled or more than doubled the normal blood cholesterol of the rats to nearly 200 mg of cholesterol/100 ml (Table I). Pretreatment with ≥10 mg of ciprofibrate/kg protected the rats from the hypercholesterolemic effects of tyloxapol. The rats also were protected from tyloxapol hypercholesterolemia by pretreatment with clofibrate at 180 mg/kg. At 90 mg/kg, clofibrate did not fully protect the rats. The clofibrate-pretreated rats had a serum cholesterol of 132.4 mg/100 ml, which was significantly above that of the normolipidemic control rats, 75.2 mg/100 ml. Accordingly, ciprofibrate may be judged to be ~18 times as effective as clofibrate in protecting rats against tyloxapol hypercholesterolemia.

Inhibition of Hormone-Sensitive Lipase—The control hormone-sensitive lipase findings (Table II) show that norepinephrine at 30 μg/kg doubled or more than doubled the serum nonesterified fatty acid level. The increases at 10 μg/kg were not significant.

In both trials, clofibrate inhibited the increase in the nonesterified fatty acid level that followed 30 μg of norepinephrine/kg. The serum nonesterified fatty acid levels of the clofibrate-pretreated rats given 10 μg of norepinephrine/kg were not significantly above control levels.

In both trials, the serum nonesterified fatty acid levels following ciprofibrate or lifibrate pretreatment were elevated to the same extent as in the control norepinephrine-treated rats. Thus, neither ciprofibrate nor lifibrate inhibited the hormone-sensitive lipase.

Serum cholesterol levels were significantly decreased by all of the hypolipidemic agents tested. The serum cholesterol levels following pretreatment with the hypolipidemic agents were all in the 35–44-mg/100 ml range, significantly less than control levels.

DISCUSSION

The hypercholesterolemic effects of tyloxapol were counteracted by pretreatment with 10 or 25 mg of ciprofibrate/kg daily or with 180 mg of clofibrate/kg daily. Pretreatment of the rats with 90 mg of clofibrate/kg daily did not protect the rats entirely from the effects of tyloxapol. This amount of clofibrate was similar to that found effective by Kariya *et al.* (4), 114 mg of clofibrate/kg (as the free acid from dietary agent at 0.25%). Thus, 10 mg of ciprofibrate/kg was approximately as protective as 180 mg of clofibrate/kg.

While ciprofibrate pretreatment was more effective in suppressing tyloxapol hypercholesterolemia than was clofibrate pretreatment, the two phenoxybutyrates were similar in that both exhibited inhibition. The protective effect noted for these agents is in accord with their ability to inhibit cholesterol biosynthesis (2, 8, 9) and with the findings that tyloxapol increases cholesterol and fatty acid synthesis in the liver (10, 11). In discussing the hyperlipidemic effects of tyloxapol, Schotz *et al.* (12) noted that tyloxapol may interfere with plasma lipoprotein catabolism.

In contrast to their similar hypocholesterolemic effects, ciprofibrate and clofibrate differed in inhibiting the hormone-sensitive lipase *in vivo*. Thorp (13) found that clofibrate (in combination with androsterone) suppressed the increase in plasma fatty acids that occurred in rats and in dogs following epinephrine administration. The findings on clofibrate are thus in accord with those of Thorp (13) in that clofibrate, at hypocholesterolemically effective doses, inhibits the norepinephrine-activated lipase.

In contrast to clofibrate, ciprofibrate and lifibrate, at hypocholesterolemically effective doses, did not inhibit the lipase. This result supports the view that ciprofibrate may be clinically similar to lifibrate rather than to clofibrate. Since lifibrate is effective in Type II hyperlipoproteinemic subjects (14), in contrast to clofibrate which is variably effective, the distinction may be therapeutically significant.

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