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Effects of hypolipidemic drugs on hepatic CoA

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Administration of the hypolipidemic drug clofibrate leads to two- to three-fold increase in the amount of total CoA in rat liver [1–3]. This increase is mainly due to an increase in free CoA-SH [1–3]. The activity of pantothenate kinase (EC 2.7.1.33)—the first enzyme in the biosynthetic pathway of CoA in mammals—is about doubled after clofibrate feeding [3, 4]. The same increase is found for the biosynthesis of CoA from pantothenate *in vitro* [3]. No change in the activity of the enzymes degrading CoA has been found in the livers of rats fed with clofibrate [3].

In the present study, some other hypolipidemic drugs have been examined for their possible effects on the metabolism of hepatic CoA. A similar change in total CoA and its metabolism as induced by clofibrate might suggest a role of CoA in the decrease in serum lipids after the administration of such drugs.

Materials and methods. CoA (chromatopure), (−)-carnitine hydrochloride and (−)-palmitoyl carnitine were purchased from P-L Biochemicals (Milwaukee, WI). Carnitine palmitoyltransferase (EC 2.3.1.21) was prepared according to Farstad *et al.* [5]. (±)-[Me-^{3}H]Carnitine was provided by the Radiochemical Centre (Amersham, U.K.) and (+)-[1-^{14}C]pantothenic acid by New England Nuclear (Boston, MA). Other chemicals were commercial products of high purity.

The following drugs were examined: bezafibrate (Boehringer Mannheim GmbH, Mannheim, F.R.G.), ciprofibrate (Sterling-Winthrop, U.K.), clofibrate (Weiders Farmasøytiske A/S, Norway), fenofibrate (Laboratoires Fournier s.a., France), pantethine (Daiichi Seiyaku Co Ltd, Japan), probucol (Dow Chemical International Inc., U.S.A.), and tiadenol (Laboratoire L. Lafon, France). All drugs were generous gifts of the manufacturers.

Commercially pelleted rat food [55% carbohydrate, 25% protein, 2.1% fat (w/w) and containing all the necessary minerals and vitamins] containing the drugs were prepared by absorbing the different drugs dissolved in acetone into the pellets. The acetone was evaporated by a stream of air, and the pellets were dried. The amount of drug in 1 kg of pelleted food was 12.4 mmoles for all the compounds examined.

Male Wistar rats weighing 100 ± 5 g at the beginning of the experiment were used. The animals were given the drugs for 9 days, and they were allowed to drink and eat *ad lib*. At the end of the experimental period the rats were weighed, and then killed by decapitation. The livers were removed and immediately weighed and homogenized in ice-cold phosphate buffer (20 mmoles/l, pH 7.2) containing EDTA (5 mmoles/l) and L-tartrate (25 mmoles/l) to counteract the degradation of CoA during the isolation procedure [3]. Aliquots of the total homogenate were frozen in a methanol/dry ice bath within 5 min after the death of the animal. Particle-free rat liver supernatant was obtained by two centrifugations—one at 34,800 *g* for 20 min, and the other of the supernatant so obtained at 48,200 *g* for 120 min. Dialysis of the particle-free supernatant was done overnight against 200 vols of potassium phosphate buffer (20 mmoles/l, pH 7.4). The mol. wt cut-off of the dialysis tubing was 12,000 (Arthur H. Thomas Co., Philadelphia, PA).

Total CoA was estimated by the CoA-dependent incorporation of radioactive carnitine into palmitoyl carnitine as described previously [6, 7]. Two samples from each total homogenate were assayed after hydrolysis of CoA esters in KOH (0.25 moles/l) for 20 min at 20°. Pantothenate kinase was assayed in the dialysed rat liver supernatant by incubation at 37° for 10 min in a medium containing (+)-[1-^{14}C]pantothenate (0.24 mmoles/l, sp. act. 8.9 mCi/mmole), ATP (10 mmoles/l), $MgCl_2$ (10 mmoles/l), dithiothreitol (2 mmoles/l, L-tartrate (10 mmoles/l), and Tris–maleate buffer (pH 6.1, 20 mmoles/l). About 2 mg of protein from particle-free rat liver supernatant was used. The product of the reaction—4′-phosphopantothenate—was then measured by paper chromatography and liquid scintillation [4]. Biosynthesis of CoA in the rat liver supernatant was estimated by incubation at 37° for 120 min with an ATP-regenerating system [3]. In addition to the contents of the assay medium for pantothenate kinase (see earlier), the medium also contained phosphocreatine (20 mmoles/l), creatine kinase (24 U/ml) and L-cysteine (0.5 mmoles/l). CoA and intermediates of CoA biosynthesis were assayed by paper chromatography followed by liquid scintillation [3]. Liver triglycerides and cholesterol were extracted with isopropanol and estimated by a Technicon Autoanalyser method [8, 9]. Carnitine palmitoyltransferase was measured as in Ref. 10. The statistical significance of the difference between groups was determined by the Wilcoxon–Mann–Whitney test [11].

Results and discussion. Table 1 shows the effect of the hypolipidemic drugs examined on the wt of the liver and on its content of CoA and protein. Clofibrate, the clofibrate analogs bezafibrate, ciprofibrate and fenofibrate, and tiadenol all caused an increase of total CoA by 1.4–2 times as compared to normal liver when related to the amount of protein, and by 2–3 times when related to the whole liver. Clofibrate gave a less pronounced increase (40%) than observed previously [3], probably due to a somewhat shorter feeding period (9 vs 10–14 days) [3]. The difference in total CoA between normal fed and each of the groups which showed elevated levels was statistically significant ($P < 0.05$). After treatment with probucol and pantethine, total CoA in rat liver was unchanged.

Bezafibrate, fenofibrate and tiadenol caused an increase in the liver wt, an effect also observed when clofibrate is given to male rats for 10–14 days (Table 1) [3, 12, 13]. This wt gain is not due to accumulation of lipids, because in none of the groups were liver triglycerides and cholesterol increased as much as to account for 1% of the liver wt increase (results not shown). The reason for the increased liver wt is mainly a hypertrophy of liver cells [14–16]. Ciprofibrate feeding resulted in a lower body wt than in control animals (Table 1), the cause for this wt reduction being unknown.

Table 2 shows the effect of the hypolipidemic drugs studied on the activity of pantothenate kinase, the first enzyme in the biosynthetic pathway of CoA in mammals, and on the biosynthesis of CoA as measured by the incorporation of (+)-[1-^{14}C]pantothenate into CoA. Table 2 also shows the activity of carnitine palmitoyltransferase measured in total homogenate from rat liver. This enzyme is confined to the mitochondrial subfraction of rat liver [17], and its sp. act. is increased about three-fold in rat liver after administration of clofibrate [3, 16–20]. There is a parallel response of increased pantothenate kinase activity and the rate of biosynthesis of CoA estimated *in vitro* during feeding with bezafibrate, ciprofibrate, fenofibrate

Table 1. Effect of hypolipidemic drugs on the content of CoA in rat liver

Group	Wt of animals† (g)	Liver wet wt	Liver total protein	Total CoA (nmoles/mg protein)
Control	175 (161–184)	8.1 (6.4–9.9)	1.0 (0.8–1.2)	1.7 (1.4–1.8)
Clofibrate	172 (163–183)	9.4 (8.5–10.6)	1.1 (0.9–1.2)	2.2 (2.0–2.3)*
Bezafibrate	176 (169–184)	12.7 (12.0–13.4)*	1.6 (1.4–1.8)*	3.3 (3.0–3.7)*
Ciprofibrate	136 (127–150)*	10.7 (9.3–12.5)	1.5 (1.3–1.8)*	2.8 (2.4–3.3)*
Fenofibrate	155 (144–163)	11.6 (10.2–13.1)*	1.6 (1.5–1.8)*	3.0 (2.4–3.4)*
Pantethine	166 (145–178)	7.6 (5.3–9.5)	0.9 (0.6–1.0)	1.6 (1.6–1.7)
Probucol	170 (152–182)	8.9 (7.2–10.6)	1.0 (0.8–1.3)	1.6 (1.4–1.7)
Tiadenol	162 (154–170)	10.8 (9.6–12.6)*	1.5 (1.3–1.7)*	3.3 (2.6–3.7)*

The table shows arithmetical mean values (range in parentheses) from two experiments with a total number of four animals in each group. CoA was estimated by the CoA-dependent incorporation of radioactive carnitine into palmitoyl carnitine [6, 7].

* $P < 0.05$ (Wilcoxon–Mann–Whitney test for independent samples compared to the control group).

† The wts of the animals at the start of the experiment were 100 ± 5 g.

Table 2. Pantothenate kinase activity and biosynthesis of CoA measured in dialyzed particle-free rat liver supernatant, and carnitine palmitoyltransferase in total homogenate of rat liver

Group	Pantothenate kinase activity (nmoles × mg protein^{-1} × min^{-1} × 10^{-1})	Biosynthesis of CoA	Carnitine palmitoyltransferase (counts × min^{-1} × mg^{-1} × min^{-1})†
Control	2.1 (1.7–2.5)	0.7 (0.6–0.7)	545–771
Clofibrate	3.0 (2.1–3.9)	1.0 (0.9–1.1)*	1250–1640
Bezafibrate	4.1 (2.8–5.7)*	1.2 (1.2–1.3)*	1321–1805
Ciprofibrate	3.6 (2.9–4.2)*	1.6 (1.4–1.7)*	1020–1376
Fenofibrate	3.4 (2.7–4.2)*	1.6 (1.3–1.8)*	1322–1956
Pantethine	2.9 (2.0–3.8)	0.8 (0.7–0.9)	529–825
Probucol	2.5 (2.2–2.8)	0.7 (0.6–0.7)	526–769
Tiadenol	3.5 (3.0–4.1)*	1.6 (1.4–1.7)*	1534–2491

CoA biosynthesis was measured by incubating dialyzed supernatants (approximately 2 mg of protein) with an ATP-regenerating system at 37° for 120 min. The medium contained [1-^{14}C]pantothenic acid [0.24 mmoles/l (sp. act. 13,000 counts × min^{-1} × nmole^{-1})], L-cysteine (0.5 mmoles/l), dithiothreitol (2 mmoles/l), $MgCl_2$ (10 mmoles/l), ATP (10 mmoles/l), phosphocreatine (20 mmoles/l) and creatine kinase (6 U) (total activity in each tube). The amount of CoA formed was estimated by counting the radioactivity in the "CoA peak" after paper chromatography [3].

Pantothenate kinase activity was assayed in the same medium omitting L-cysteine, phosphocreatine and creatine kinase. The incubation lasted for 10 min at 37°. Carnitine palmitoyltransferase was measured as in Ref. 10. The numbers are mean values for four animals (range in parentheses).

* $P < 0.05$ (Wilcoxon–Mann–Whitney test for independent samples compared to the control group).

† Results shown for two animals in each group. (The activity of carnitine palmitoyltransferase is assayed as an exchange reaction and therefore given in arbitrary units.)

and tiadenol. After clofibrate treatment these changes were less pronounced, and reached the 5% level of significance only for the biosynthesis of CoA. However, the increased activity of pantothenate kinase after clofibrate feeding for 10–14 days has been well established previously [3, 4]. The lower activity of pantothenate kinase after clofibrate administration for 9 days may reflect a lower potency of clofibrate than of bezafibrate, ciprofibrate, fenofibrate and tiadenol in this respect. In general, parallel changes of total CoA, the biosynthesis of CoA, the activity of pantothenate kinase and carnitine palmitoyltransferase were observed with the different drugs (Tables 1 and 2). As the animals had free access to food and water, the amount of drug ingested each day may have varied slightly. However, the wt gain in all the groups, except in the ciprofibrate group, is comparable (Table 1). We estimated the intake of rat pellets to be about 15 g in 24 hr. This corresponds to about 185 μmoles of drug ingested. For clofibrate this amount is equivalent to 335 mg × kg body wt^{-1}. This is one order of magnitude higher than the recommended dosage for clofibrate when used as a serum lipid lowering agent in man, i.e. 2 g daily (30 mg × kg body wt^{-1}) [21]. However, the drug dosage used here is the same as used by others in animal experiments [1, 15–20, 22].

The similarity of the effects of clofibrate, its analogs and tiadenol on the parameters shown in Tables 1 and 2 points to a role for CoA in the mechanism(s) leading to lowered serum lipids, and in particular serum triglycerides [21], after administration of these drugs. An increased concn of CoA may influence fatty acid activation, which is increased after clofibrate feeding [16], and act on the chemical equilibrium: CoA-SH + long-chain acylcarnitine ⇆ carnitine + long-chain acyl-CoA catalysed by carnitine palmitoyltransferase. The transport of acylcarnitine into mitochondria is likely to be stimulated by the increased activity of carnitine palmitoyltransferase (Table 2) [3, 16–20, 23]. After clofibrate administration, mitochondrial CoA is increased relatively more than cytosolic CoA [3]. This may, by the above reaction, increase the rate of formation of mitochondrial acyl-CoA from incoming acylcarnitine. Increased capacity for β-oxidation of long-chain acyl-CoA furnished by this reaction has been demonstrated in hepa-

tocytes isolated from clofibrate-fed rats [24].

The drugs probucol and pantethine did not affect the parameters of the metabolism of hepatic CoA shown in Tables 1 and 2. In contrast to the remaining drugs, probucol does not lower the level of total triglycerides in serum when given to humans, but may lower LDL-cholesterol by 10–15% [21]. This relationship may also suggest that the effect on the metabolism of CoA is related to the triglyceride lowering effect of clofibrate, the clofibrate analogs and tiadenol.

Summary. The hypolipidemic drugs bezafibrate, ciprofibrate, clofibrate, fenofibrate, pantethine, probucol and tiadenol were supplied during 9 days to male rats weighing 100 g. The drug dosage was about 185 μmoles in 24 hr. Increased biosynthesis and content of CoA, and increased activity of pantothenate kinase and carnitine palmitoyltransferase were found in the livers of rats fed bezafibrate, ciprofibrate, clofibrate, fenofibrate and tiadenol. A role of CoA-SH in the serum triglyceride lowering mechanism(s) of these drugs is discussed.

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REFERENCES

1. S. Miyazawa, T. Sakurai, M. Imura and T. Hashimoto, *J. Biochem., Tokyo* **78**, 1171 (1975).
2. M. J. Savolainen, V. P. Jauhonen and I. E. Hassinen, *Biochem. Pharmac.* **26**, 425 (1977).
3. S. Skrede and O. Halvorsen, *Eur. J. Biochem.* **98**, 223 (1979).
4. O. Halvorsen and S. Skrede, *Eur. J. Biochem.* **124**, 211 (1982).
5. M. Farstad, J. Bremer and K. R. Norum, *Biochem. biophys. Acta* **132**, 492 (1967).
6. S. Skrede, *Eur. J. Biochem.* **38**, 401 (1973).
7. S. Skrede and J. Bremer, *Eur. J. Biochem.* **14**, 465 (1970).
8. J. B. Levine and B. Zak, *Clinica Chim. Acta* **10**, 381 (1964).
9. G. Kessler and H. Lederer, in *Automation in Analytical Chemistry* (Ed. L. T. Skeggs, Jr), p. 341. Technicon Symp. Mediad. Inc., New York (1966).
10. K. R. Norum and J. Bremer, *J. Biol. Chem.* **242**, 407 (1967).
11. D. Colquhoun, in *Lectures in Biostatistics*, p. 143. Clarendon Press, Oxford (1971).
12. M. M. Best and C. H. Duncan, *J. Lab. clin. Med.* **64**, 634 (1964).
13. R. Hess, W. Staübli and W. Reiss, *Nature, Lond.* **208**, 856 (1965).
14. G. E. Paget, *J. Atheroscler. Res.* **3**, 729 (1963).
15. G. Kolde, A. Roessner and H. Themann, *Virchows Arch. B. Cell Path.* **22**, 73 (1976).
16. L. N. W. Daae and M. Aas, *Atherosclerosis* **17**, 389 (1973).
17. M. T. Kahönen, *Biochim. biophys. Acta* **428**, 690 (1976).
18. H. E. Solberg, M. Aas and L. N. W. Daae, *Biochim. biophys. Acta* **280**, 434 (1972).
19. H. E. Solberg, *Biochim. biophys. Acta* **360**, 101 (1974).
20. M. A. K. Markwell, L. L. Bieber and N. E. Tolbert, *Biochem. Pharmac.* **26**, 1697 (1977).
21. R. I. Levy, in *The Pharmacological Basis of Therapeutics* (Eds. A. Goodman Gilman, L. S. Goodman and A. Gilman), p. 834. Macmillan, New York (1980).
22. P. B. Lazarow and C. de Duve, *Proc. natn. Acad. Sci. U.S.A.* **73**, 2043 (1976).
23. H. Voltti and I. E. Hassinen, *Life Sci.* **28**, 47 (1981).
24. R. Z. Christiansen, *Biochim. biophys. Acta* **530**, 314 (1978).

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Demonstration of dipeptidyl carboxypeptidase activity in rat brain stem synaptosomes using first-order kinetic analysis

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Dipeptidyl carboxypeptidase (DCP; angiotensin-converting enzyme; kininase II; EC 3.4.15.1) is a ubiquitously distributed enzyme that cleaves dipeptide residues from the carboxyl terminal of distinct peptides. It is involved in the synthesis of angiotensin II and the catabolism of bradykinin [1]. There is also a growing body of evidence that indicates that DCP might be responsible for the synthesis [2, 3] or degradation [4–6] of met- and leu-enkephalin. Although the presence of DCP has been reported in various brain regions [7–11], the subcellular localization of this enzyme in distinct brain areas has not been studied in detail. Yang and Neff [7] reported that 32% of whole brain DCP was localized to the crude mitochondrial fraction, which contains a number of organelles, including nerve terminals [12]. Arregui *et al.* [9], on the basis of intrastriatal kainic acid lesions, concluded that DCP in the substantia nigra was localized primarily to nerve endings. However, to our knowledge, direct localization of DCP to enriched synaptosomes, which are believed to be composed primarily of nerve terminals [12], has not been reported in the literature. Furthermore, the precise role of DCP in brain is unknown, and it is also unclear whether DCP is specific for a few biologically active peptides or whether it is a general, non-specific peptidase. Recently, Chevillard and Saavedra [11] reported a heterogenous distribution of DCP in rat brain stem. This uneven distribution of DCP is consistent with the hypothesis that the enzyme participates in the processing of as yet unidentified, but biologically