RAPID FEEDBACK INHIBITION OF ENDOGENOUS CHOLIC AND CHENODEOXYCHOLIC ACID SYNTHESIS BY EXOGENOUS CHENODEOXYCHOLIC ACID IN MAN*

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SUMMARY

Chenodeoxycholic acid (300 mg + ^{14}C) was administered orally to a bile fistula patient receiving a constant infusion of {^{3}H}mevalonic acid. Suppression of endogenous cholic and chenodeoxycholic acid synthesis occurred within 2 to 4 hours and continued for the next 10 hours; synthesis returned to the baseline level after 18 hours. Incorporation of {^{3}H}mevalonic acid into both bile acids was also greatly reduced during the first several hours after chenodeoxycholic acid, but almost recovered by 5 hours. The data suggest that multiple feedback sites are involved in the regulation of bile acid synthesis in man.

It has been demonstrated (1-6) that the enzymes HMG-CoA reductase and 7α-hydroxylase which catalyze the conversion of HMG-CoA to mevalonic acid and the 7α-hydroxylation of cholesterol are the feedback sites on which bile acids act to regulate their own synthesis and that of their obligatory cholesterol precursor. These findings have been made predominantly in the rat, but recent reports (7,8) have shown that the regulation of HMG-CoA reductase, synthesis is probably intimately related to the control of cholesterol synthesis in man. The limited information which is available on the in vivo regulation of bile acid synthesis in man have been obtained in conjunction with the long term feeding of chenodeoxycholic acid to solubilize cholesterol gallstones. It has been shown, using the isotope dilution technique that chenodeoxycholic acid fed to patients with gallstones for 6 months to a year markedly inhibits cholic acid synthesis (9) No information has been obtained on the effect of administered chenodeoxycholic

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acid on its own synthesis, since the isotope dilution technique cannot be used to measure endogenous synthesis following the administration of the same bile acid. In the present report, data are provided for the first time in man on the immediate (hours) effect of administered chenodeoxycholic acid on the endogenous synthesis of both cholic and chenodeoxycholic acid and its possible site(s) of action.

MATERIAL AND METHODS

Patient. The patient used was a 56 year old white male who had a Baldwin T-tube inserted in the common duct following cholecystectomy and a common duct exploration for cholesterol gallstones. The experiment was carried out 3 weeks after surgery. The T-tube was open for 7 days prior to the experiment, and the patient's bile flow averaged 900 ml/day. Complete collection of bile during the experiment was assured by the inflation of a balloon in the distal arm of the T-tube and verified by x-ray. A constant infusion of {5-^{3}H}mevalonic acid (16 μCi/hr) was started in the morning (9 a.m.) and was maintained at the same rate for 9 hours. Four hours after the start of the infusion, the patient was administered via nasogastric tube 300 mg of chenodeoxycholic acid plus 8 μCi {^{14}C}-chenodeoxycholic acid. Bile was collected at 20 minute intervals throughout the experiment.

Labeled Compounds. The DL-{5-^{3}H}mevalonic acid (DBED salt) was obtained from New England Nuclear Corp. and liberated from the salt by the addition of sodium bicarbonate followed by ethyl-ether extraction of DBED. The aqueous solution of sodium {^{3}H}mevalonate was neutralized with an equimolar amount of HCl and diluted with sterile normal saline to a volume of 50 ml. The solution was passed through a millipore filter (0.22μ) and administered intravenously to the patient. The {24-^{14}C}chenodeoxycholic was obtained from ICN Pharmaceuticals, Inc. and checked for purity (97%) by thin layer chromatography on silica gel G using a solvent system of isooctane-ethyl acetate-acetic acid 10:10:2.

Methods. Bile was extracted with 20 volumes of 2:1 chloroform methanol. The chloroform phase was analyzed for phospholipid by the method of Bartlett (10) and for cholesterol by the method of Sperry and Webb (11). Bile acids were

determined by a combination of thin layer and gas liquid chromatography as described earlier (12). The ^{14}C and ^{3}H activities of the bile acid fractions were determined in a Mark III liquid scintillation counter (Searle Analytic) and corrected for quench by the external standard method.

Design of the Experiment. The T-tube patient was used for studying the effect of chenodeoxycholic acid on endogenous bile acid synthesis since in this model: a) bile acid secretion is equal to bile acid synthesis, b) the patient studied had a constant bile acid secretion rate implying a steady state, and c) the effect of administered bile acids on endogenous bile acid synthesis and possible feedback site(s) could be ascertained by determining simultaneously the secretion of bile acids and the incorporation of {^{3}H}mevalonic acid into the bile acids. The administration of {^{14}C}chenodeoxycholic acid of known specific activity allowed for the direct determination of endogenous chenodeoxycholic acid synthesis by simple isotope dilution since the bile fistula patient does not have a bile acid pool. Significant deviation from the buildup of ^{3}H activity in the bile acids following {^{3}H}mevalonic acid infusion allowed for an evaluation of the possible site(s) of action of chenodeoxycholic acid on the rate limiting steps of cholesterol and/or bile acid synthesis. The incorporation of ^{3}H activity into cholesterol should not have been effected since there is no evidence for rate limiting steps beyond mevalonic acid. Significant depression of radioactivity into both bile acids could therefore be indicative of a suppression of the 7α-hydroxylation step in the bile acid pathway. Alternatively, a greater decrease in the mass of bile acid synthesized relative to the incorporation of ^{3}H into bile acids could indicate a greated effect of chenodeoxycholic acid on cholesterol synthesis.

RESULTS

Fig. 1 shows the effect of the chenodeoxycholic acid on endogenous bile acid synthesis. The secretion of cholic and chenodeoxycholic acids was relatively constant prior to the administration of chenodeoxycholic acid. Exogenous chenodeoxycholic acid rapidly appeared in bile and reached a peak in 2 hours and then

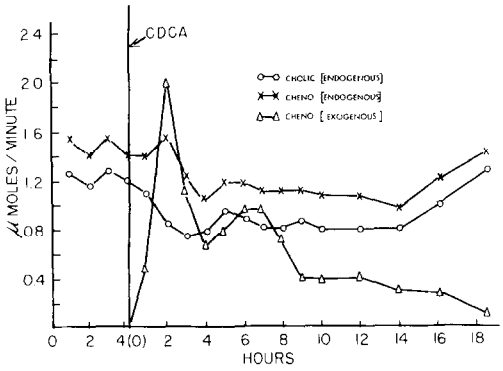

Fig. 1. Effect of exogenous chenodeoxycholic acid (CDCA) on the secretion of endogenous bile acids. O—O = cholic acid (endogenous) X—X = chenodeoxycholic acid (endogenous Δ—Δ = chenodeoxycholic acid (exogenous).

declined. Recovery of administered {^{14}C}chenodeoxycholic acid activity was 70% in 8 hours and 92% for the total 18 hour period. Endogenous cholic and chenodeoxycholic acid synthesis were rapidly suppressed within the first 4 hours after chenodeoxycholic acid administration. While cholic acid synthesis was inhibited within 1 to 1.5 hours, there was a 1.5 to 2 hour delay in the inhibition of chenodeoxycholic acid synthesis. The depression of bile acid synthesis continued until the 14th hour and then started to increase so that by the 18th hour the synthesis of both bile acids had returned to the original baseline level. During the 4 to 14 hour period after chenodeoxycholic acid, the average decrease in endogenous cholic and chenodeoxycholic acids was 32% and 29% respectively. These decreases were found to be significant ($P < 0.01$) when compared to the control period.

Fig. 2 shows the effect of chenodeoxycholic acid on the incorporation of ^{3}H activity into the newly synthesized bile acids. Following 4 hours of {^{3}H}-mevalonic acid infusion the output of ^{3}H activity in bile acids tended to reach a plateau. Within 1.5 to 2 hours following the administration of chenodeoxycholic there was a marked depression of ^{3}H activity into both bile acids which averaged 40% for cholic and 60% for chenodeoxycholic acid. During the next 3.5 hours there was a gradual return of ^{3}H incorporation into both bile acids.

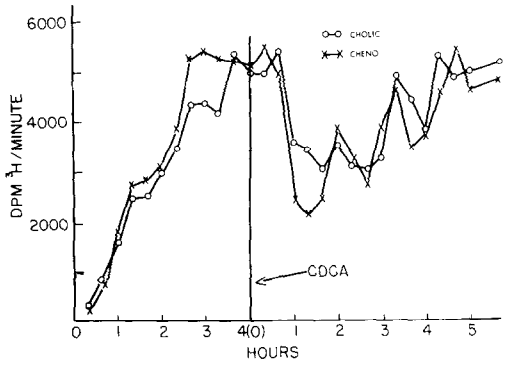

Fig. 2. Effect of exogenous chenodeoxycholic acid (CDCA) on the incorporation of {^{3}H}mevalonic acid into bile acids. Values for cholic acid were corrected for random loss (8.33%) of ^{3}H activity at position 12 of cholesterol during conversion to bile acids. O—O = cholic acid X—X = chenodeoxycholic acid.

Fig. 3 shows the effect of chenodeoxycholic acid on the secretion of biliary phospholipid, cholesterol, and total bile acids. The secretion of the biliary lipids was relatively constant prior to the administration of chenodeoxycholic acid. The total bile acid peaked at 2 hours after the administration of chenodeoxycholic acid followed by a second rise at 6 hours. Phospholipid secretion paralleled the total bile salts, but there was no appreciable change in cholesterol secretion.

DISCUSSION

The data of the present report have shown that the administration of a small dose (300 mg) of chenodeoxycholic acid to man markedly inhibited the endogenous synthesis of both cholic and chenodeoxycholic acids. The rapidity of this suppressive effect and the continued inhibition of both bile acids over a 10 hour period suggests the presence of one or more feedback site(s) in the liver which is common (at least in part) to the pathway of both cholic and chenodeoxycholic acids. The initial rapid phase of bile acid inhibition is consistent with a change in the state of the enzyme protein on an allosteric type of feedback inhibition. Inhibition of bile acid synthesis continued after 4 hours even though a major portion of the administered chenodeoxycholic acid had been eliminated in the bile. Since {^{3}H}

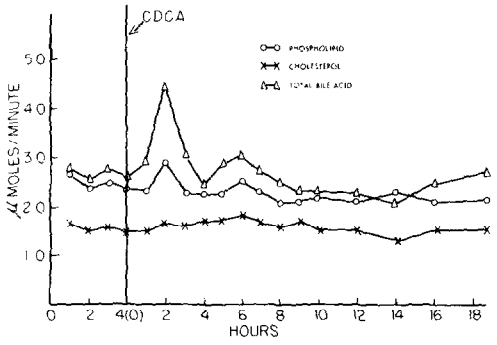

Fig. 3. Effect of chenodeoxycholic acid (CDCA) on biliary lipid secretion. O—O = phospholipid X—X = cholesterol Δ—Δ = total bile acids.

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mevalonic acid incorporation into cholesterol is probably not effected by the administered chenodeoxycholic acid, it seems likely that inhibition of the 7α-hydroxylation step in the bile acid pathway is partly responsible for the reduced labeling of the endogenous bile acids which occurred during the first 2 hours. However, in the later phase of the experiment, there was a recovery of incorporation of ^{3}H radioactivity into the bile acids even though there was a continuous inhibition of bile acids synthesis. If the observed recovery of ^{3}H activity in the bile acid fraction was solely caused by a return of 7α-hydroxylase activity, then the mass of endogenous bile acids should have increased in a parallel fashion. Consequently, the dissociation between endogenous bile acid synthesis and the incorporation of {^{3}H}mevalonic acid into both bile acids suggests that after the initial suppression of 7α-hydroxylase an additional feedback site was effected. This inhibition could have been at the level of HMG-CoA reductase which would have resulted in a decreased availability of newly synthesized cholesterol for bile acid synthesis (13). Recovery of ^{3}H activity in the bile acid fraction could occur in the presence of a continuous inhibition of 7α-hydroxylase activity since even a reduced amount of enzyme would be sufficient to hydroxylate the smaller amount of higher specific activity cholesterol substrate. The prolonged suppression of endogenous bile acid synthesis, after most of the exogenous chenodeoxycholic

acid had been eliminated in the bile and the time delay necessary to invoke a secondary feedback effect are consistent with a reduction in the synthesis of the enzyme HMG-CoA reductase (14). The data also suggest that the mechanism of chenodeoxycholic and cholic acid feedback inhibition may not be entirely similar since cholic acid synthesis was suppressed initially to a greater extent than chenodeoxycholic acid.

The effect of chenodeoxycholic acid on biliary lipid secretion is consistent with the earlier findings (15) in man and indicates that phospholipid secretion is closely linked to the influx of bile acids into the liver. Cholesterol secretion is more independent of changes in bile acid concentration and was not significantly altered by chenodeoxycholic acid.

REFERENCES

1. Siperstein, M.D., and Fagan, V.J., J. Biol Chem. 241, 602 (1966).
2. Shefer, S., Hauser, S., Bekersky, I., and Mosbach, E.H., J. Lipid Res. 11, 404 (1970).
3. Mosbach, E.H., Arch. Intern. Med. 130, 477 (1972).
4. McNamara, D.J. and Rodwell, V.W., In Biochemical Regulatory Mechanisms in the Eucaryotic Cell, 205-242, 1972, John Wiley and Sons, New York, N.Y.
5. Hampretch, B., Nüssler, C., Waltinger, G., and Lynen, F., Eur. J. Biochem. 18, 10 (1971).
6. Shapiro, D.J. and Rodwell, V.W., J. Biol. Chem. 246, 3210 (1971).
7. Goldstein, J.L. and Brown, M.S., Proc. Nat. Acad. Sci. 70, 2804 (1973).
8. Fogelman, A.M., Edmond, J., Seager, J., and Popjak G., J. Biol. Chem. 250, 2045 (1975).
9. Danzinger, R.G., Hofmann, A.F., Thistle, J.L., and Schoenfield, L.J., J. Clin. Invest. 52, 2809 (1973).
10. Bartlett, G.R., J. Biol. Chem. 234, 466 (1959).
11. Sperry, W.M. and Webb, J., J. Biol. Chem. 187, 97 (1950).
12. Vlahcevic, Z.R., Bell, C.C., Jr., Buhac, I., Farrar, J.T., and Swell, L., Gastro. 59, 165 (1970).
13. Balasubramaniam, S., Mitropoulos, K.A., Myant, N.B., Eur. J. Biochem. 34, 77 (1973).
14. Higgins, M.J.P., Brady, D., and Rudney, H., Arch. Biochem. Biophys. 163, 271 (1974).
15. Bell, C.C., Jr., Vlahcevic, Z.R., and Swell, L., Surg. Gyn. and Obst., 132, 36 (1971).