EFFECT OF FOOD DEPRIVATION AND DRUG ADMINISTRATION ON INTESTINAL ESTERASE ACTIVITY

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Abstract—Esterase activity, and contents of protein, phospholipid, and cholesterol were measured in the liver and intestinal mucosa of male rats after 24 hr of food deprivation or treatment with corn oil (2.5 ml/kg, 3 days), aspirin (500 mg/kg, 3 days), cinchopen (600 mg/kg, 3 days), or phenobarbital (100 mg/kg, 3 days). After food deprivation for 24 hr, the total esterase activity was decreased in the intestinal mucosa and liver. Treatment with corn oil, increased the mucosal esterase activity to about twice that of the control but hepatic esterase activity did not change markedly, while total activity was decreased in post-mitochondrial supernatant or microsomal fraction of intestinal mucosa by treatment with cinchopen. Total and specific activities were increased in lysosomal fraction of intestinal mucosa by treatment with aspirin or cinchopen. In these rats, hepatic esterase activity was not markedly changed. In rats treated with corn oil or phenobarbital, cholesterol content in the intestinal mucosa was decreased, and the phospholipid content was increased significantly. By food deprivation or treatment with cinchopen, the content of protein, phospholipid or cholesterol decreased. These results indicate that characteristics of the esterase activity of intestinal mucosa are quite different from that in the liver. The dietary factor and drug administration may interfere with the intestinal ester-hydrolysis capacity.

Recently, a variety of ester-type drugs is being used in many ways as modified types of antibiotics and hormonal drugs for increasing absorbability, permeability, and other properties of drugs. In addition to the liver, many extrahepatic tissues including the small intestine are able to transform drugs and other foreign compounds to their metabolites (1–3). Ester-type drugs are absorbed by the intestinal mucosa after being hydrolyzed by esterases which are bound in the intestinal mucosa. The degree of hydrolysis affects pharmacological activity or toxicity of ester-type drugs (4–5). It is known that the activity of drug metabolizing enzymes in the liver is influenced by various physiological conditions or by drug administration (6–7). The mono-oxygenase system and glucuronidation activity of the intestine are influenced by food deprivation and treatment with drugs, but to our knowledge there are no reported data on esterases of the intestinal mucosa (8–9). The present study was done to elucidate the role of dietary factors or drug administration in the regulation of the mucosal rate of drug hydrolysis and its effect on the inducibility of esterases. For this purpose, rats were deprived of food or administered various drugs, and correlations among esterase activity and contents of protein, phospholipid or cholesterol in subcellular fraction were examined.
MATERIALS AND METHODS

Administration of drugs and food deprivation

Male albino rats of Wistar strain, weighting 200-250 g were used. Control and drug-administered rats had free access to pelleted rat food (Oriental Co.) and tap water. Aspirin (500 mg/kg), cinchophen (600 mg/kg) or phenobarbital (100 mg/kg), or corn oil (2.5 ml/kg) was administered intragastrically for 3 days. Other rats were completely deprived of food for 24 hr.

Preparation of intestinal mucosa and liver

The rats were sacrificed by a blow on the head and exsanguinated by cutting the renal vessels. Liver and intestine were dissected at 4°C. The small intestine was cut into 10 cm segments. These mucosae were scraped off with a glass slide and homogenized in a Potter-Elvehjem type Teflon homogenizer in Tris-mannitol medium (0.01 M Tris-HCl buffer (pH 7.4), 0.278 M mannitol). The liver was homogenized in 1.15% KCl.

Homogenates of the liver and intestinal mucosa were centrifuged at 10,000 g for 10 min, its supernatant at 20,000 g for 20 min, and the pellet at 105,000 g for 60 min, to obtain the post-mitochondrial supernatant (I fraction), lysosomal fraction (II fraction), and microsomal fraction (Mic fraction), respectively.

Assays

The protein was determined by the method of Lowry et al. (10). The amount of phospholipid was determined by measuring the inorganic phosphate after hydrolysis with perchloric acid as described by Chen et al. (11), and the results were expressed as equivalent to lecithin. Cholesterol content of I, II, or Mic fraction was measured as described by Franey and Amader (12). Esterase activity was assayed using p-nitrophenyl acetate as a substrate (13).

RESULTS

Changes in protein, phospholipid, and cholesterol contents of subcellular fractions

a. Intestinal mucosa

In I and Mic fractions, the contents of protein, phospholipid, and cholesterol were decreased by treatment of the rats with cinchophen. In rats deprived of food, the contents of protein, phospholipid, and cholesterol were decreased when calculated on 100 g body weight basis. The protein content of these fractions did not change markedly in rats treated with corn oil or aspirin. In rats treated with corn oil or phenobarbital, the cholesterol content was decreased significantly, after which the phospholipid content was increased significantly (Table 1).

b. Liver

In liver microsomes, the contents of protein, phospholipid, and cholesterol did not change markedly by treatment with corn oil or aspirin. In rats treated with phenobarbital, the contents of protein, phospholipid, and cholesterol were increased significantly (Table 1).

Esterase activity

In I or Mic fraction of intestinal mucosa in rats deprived of food, the total activity
was decreased, but the specific activity showed control level. The decrease was approximately proportional to the decrease in mucosal protein. Total esterase activity in hepatic

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Fig. 1. Effect of food deprivation, corn oil and drug administration on esterase activity of intestinal mucosa. I: 10,000 g supernatant, II: 20,000 g pellet, Mic: 10,5000 g pellet. Vertical bars represent S.E. for five experiments, Symbols as in Table 1.

Fig. 2. Effect of food deprivation, corn oil and drug administration on esterase activity of hepatic microsomes. Con: Control, Sta: Starvation, Oil: corn oil, Asp: Aspirin, Cin: Cinchophen, Phe: Phenobarbital, Symbols as in Table 1.
| Table 1. Protein, cholesterol and phospholipid contents of intestinal mucosa and hepatic microsomes after food deprivation or drug administration |
|---|---|---|---|---|
| | Control | L Mic | I I | Food deprivation |
| Protein | | | | |
| mg/100 g b.w. | 15.93 ± 2.43 | 2.46 ± 0.32 | 127.23 ± 15.33 | 5.13 ± 1.57** | 1.30 ± 0.21** | 177.42 ± 25.33* |
| mg/g | 42.98 ± 2.14 | 7.11 ± 0.49 | 24.90 ± 3.95 | 53.93 ± 2.44** | 7.99 ± 0.35* | 24.00 ± 2.32 |
| Cholesterol | | | | |
| mg/100 g b.w. | 1.69 ± 0.24 | 0.47 ± 0.09 | 10.22 ± 1.85 | 1.35 ± 0.11 | 0.27 ± 0.12* | 8.08 ± 0.66 |
| mg/g | 4.53 ± 0.54 | 0.76 ± 0.12 | 2.00 ± 0.34 | 7.25 ± 0.34** | 0.92 ± 0.09** | 2.54 ± 0.45 |
| mg/mg protein | 0.13 ± 0.02 | 0.11 ± 0.02 | 0.08 ± 0.01 | 0.13 ± 0.01 | 0.10 ± 0.03 | 0.11 ± 0.06 |
| Phospholipid | | | | |
| mg/100 g b.w. | 0.12 ± 0.05 | 0.03 ± 0.01 | 29.64 ± 5.64 | 0.06 ± 0.02** | 0.01 ± 0.01* | 19.38 ± 3.42** |
| mg/g | 0.36 ± 0.03 | 0.13 ± 0.02 | 5.80 ± 1.32 | 0.62 ± 0.07** | 0.20 ± 0.03** | 6.03 ± 0.79 |
| mg/mg protein | 0.008 | 0.015 | 0.270 | 0.011 | 0.013 | 0.250 |
| ±0.002 | ±0.003 | ±0.054 | ±0.001 | ±0.002 | ±0.065 |

| Protein | 11 | I Mic | L Mic | I I | Food deprivation |
|---|---|---|---|---|---|
| Control | | | | | |
| mg/100 g b.w. | 15.36 ± 2.22 | 2.11 ± 0.34 | 136.84 ± 23.45 | 14.73 ± 1.55 | 2.42 ± 0.43 | 115.32 ± 23.15 |
| mg/g | 45.80 ± 5.76 | 6.56 ± 0.43 | 27.32 ± 2.41 | 40.98 ± 2.14 | 7.53 ± 0.49 | 23.90 ± 1.95 |
| Cholesterol | | | | | | |
| mg/100 g b.w. | 0.71 ± 0.71** | 0.33 ± 0.09** | 12.23 ± 1.75 | 1.72 ± 0.23 | 0.52 ± 0.21 | 11.02 ± 3.42 |
| mg/g | 2.44 ± 0.34** | 0.45 ± 0.08** | 2.48 ± 0.65 | 3.91 ± 0.34 | 0.61 ± 0.07 | 2.24 ± 0.44 |
| mg/mg protein | 0.07 ± 0.01* | 0.09 ± 0.01* | 0.19 ± 0.04 | 0.11 ± 0.03 | 0.13 ± 0.02 | 0.08 ± 0.01 |
| Phospholipid | | | | | | |
| mg/100 g b.w. | 0.23 ± 0.05 ** | 0.06 ± 0.01** | 26.63 ± 4.43 | 0.13 ± 0.04 | 0.03 ± 0.01 | 27.03 ± 5.44 |
| mg/g | 0.64 ± 0.11** | 0.20 ± 0.04** | 5.40 ± 0.54 | 0.38 ± 0.03 | 0.13 ± 0.02 | 5.40 ± 0.55 |
| mg/mg protein | 0.022 | 0.025 | 0.236 | 0.010 | 0.014 | 0.254 |
| ±0.003** | ±0.006** | ±0.065 | ±0.002 | ±0.003 | ±0.044 |
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|                    | Protein          | Cinchophen | L Mic | II | L Mic | Phenobarbital | L Mic |
|--------------------|------------------|------------|-------|----|-------|---------------|-------|
|                    | mg/100 g b.w.    | 7.97 ± 2.11** | 1.50 ± 0.34** | 163.84 ± 31.22* | 16.23 ± 3.21 | 2.09 ± 0.26 | 205.23 ± 34.45** |
|                    | mg/g             | 26.40 ± 2.54** | 4.40 ± 0.87** | 32.30 ± 3.45* | 46.72 ± 4.78 | 6.03 ± 0.76 | 33.75 ± 5.64** |
| Cholesterol        | mg/100 g b.w.    | 0.83 ± 0.03** | 0.30 ± 0.02** | 8.70 ± 1.32* | 0.62 ± 0.02** | 0.22 ± 0.02** | 23.14 ± 3.54** |
|                    | mg/g             | 1.74 ± 0.02** | 0.31 ± 0.03** | 1.73 ± 0.24* | 1.77 ± 0.05** | 0.61 ± 0.03* | 3.80 ± 0.75** |
|                    | mg/mg protein    | 0.09 ± 0.02* | 0.08 ± 0.01* | 0.06 ± 0.01* | 0.05 ± 0.01** | 0.07 ± 0.01* | 0.11 ± 0.02* |
| Phospholipid       | mg/100 g b.w.    | 0.06 ± 0.01** | 0.01 ± 0.01** | 22.53 ± 3.44 | 0.17 ± 0.02* | 0.04 ± 0.01 | 38.87 ± 4.87** |
|                    | mg/g             | 0.22 ± 0.04** | 0.08 ± 0.01** | 4.40 ± 0.76 | 0.52 ± 0.08** | 0.12 ± 0.03 | 6.26 ± 1.55** |
|                    | mg/mg protein    | 0.005 | 0.011 | 0.154 | 0.011 | 0.020 | 0.288 |

1 I: Intestinal 10,000 g supernatant, 1 Mic: Intestinal microsomes, L Mic: Hepatic microsomes
Each value represents the mean ± S.E. of five experiments.
**Statistically different (p<0.01) from the control group, *Statistically different (p<0.05)
microsomes was decreased (Figs. 1b and 2).

In rats treated with corn oil, hepatic esterase activity in the Mic fraction did not change markedly, but esterase activity of the mucosa was increased about twice that of the control when calculated as a specific activity or on a 100 g body weight basis (Figs. 1c and 2).

In rats treated with aspirin, the total or specific activity in I or Mic fraction of intestinal mucosa did not change markedly but these activities in II fraction were increased significantly. In rats treated with cinchophen, total activity was decreased significantly in I, II, or Mic fraction of the intestinal mucosa (Fig. 1e). In rats treated with aspirin or cinchophen, esterase activities of hepatic microsomes did not change markedly (Fig. 2).

In rats treated with phenobarbital, the total esterase activity in I or Mic fraction of intestinal mucosa was increased (Fig. 1f). In these rats, the total or specific activity of hepatic microsomes was increased significantly (Figs. 1 and 2).

**DISCUSSION**

Mucosa of the intestine possesses drug metabolizing activity before these agents reach the systemic circulation (14, 15). The proportions of drug-metabolizing enzyme activities in extrahepatic tissues vary from species to species (2, 16). It was shown earlier that the activity of the mono-oxygenase system and glucuronidation are controlled partially through a different mechanism (2, 17).

The main components of the membrane are protein and lipid. The protein fraction has a dual function in the membrane, having both catalytic and structural properties (18, 19). The membraneous phospholipids are structural and are storage components, but they also play a role in the regulation of enzyme activities and the control of membrane permeability (19-21). Cholesterol stabilizes molecular structures, regulates protein-phospholipid interaction, contributes to membrane permeability, and regulates enzyme activities through such effects in the liver and intestine (21-23).

The esterase activity in the intestinal mucosa of rats showed a tendency to increase by treatment with corn oil, and esterase activity of Mic fraction was twice that of control values (Fig. 1c). At the same time, the cholesterol content was decreased, phospholipid content was increased, and there was no change in protein content (Table 1). From these results, the increase in esterase activity may be related to fluidity of the membrane. On the other hand, esterase activity in hepatic microsomal fractions was not influenced by treatment of the animals with corn oil (Fig. 2). Therefore, hepatic and intestinal esterases may indeed be controlled by different mechanisms.

Morphological changes in the intestinal mucosa during food deprivation have also been described (24), and food deprivation has been reported to decrease drug metabolism in rats in vivo (9). Our results were consistent with these observations; ester-hydrolysis capacities of the liver and intestinal mucosa were generally decreased by food deprivation.

Cinchophen blocks protein synthesis in cell-free preparations (25), and a possible inhibition of enzyme biosynthesis may follow, as total esterase activity in the intestinal mucosa was decreased by treatment of rats with cinchophen (Fig. 1e).
Intestinal esterases consist of two types, lysosomal esterase and microsomal esterase (26). In the present experiment, fraction II may have contained microsomes, but by treatment with aspirin or cinchophen, the esterase activity of II fraction was increased, while the esterase activity of Mic fraction showed no change. Therefore, we consider that the factor controlling lysosomal esterase is different from that of microsomal esterase.

The evidence herein showed that intestinal esterase activity decreased with food deprivation and cinchophen administration, and increased when corn oil or phenobarbital were administrated. The dietary factor and drug administration may interfere with intestinal ester-hydrolysis capacity. The change in intestinal esterase activity may be closely related with changes in cholesterol and phospholipid content.

REFERENCES

1) Vainio, H. AND Hietanen, E.: Biochim. Biophys. Acta 362, 92 (1974)
2) Hietanen, E. AND Vainio, H.: Acta pharmacol. toxicol. 33, 57 (1973)
3) Wattenberg, L.W., Leong, J.L. AND Strand, P.J.: Cancer Res. 22, 1120 (1962)
4) Dahl Ne, W.: J. med. Chem. 13, 357 (1970)
5) Masuda, Y.: Chemotherapy 22, 357 (1974)
6) Conney, A.H.: Pharmacol. Rev. 19, 317 (1967)
7) Kato, R. AND Gillette, J.R.: J. Pharm. exp. Ther. 150, 279 (1965)
8) Hietanen, E.M., Laitinen, M., Vainio, H. AND Hanninen, O.: Lipid 10, 467 (1975)
9) Marsilos, M. AND Laitinen, M.: Biochem. Pharmacol. 24, 1529 (1975)
10) Lowry, O.H., Rosebrough, N.J., Farr, A.L. AND RANDALL, R.J.: J. biol. Chem. 193, 265 (1951)
11) Chen, P.S., Toribara, T.Y. AND Warner, H.: Analyt. Chem. 28, 756 (1956)
12) Franey, R.J. AND Amader, E.: Clin. Chem. Acta 21, 255 (1968)
13) Morikawa, M., Inoue, M. AND Tsuboi, M.: Chem. Pharm. Bull., Tokyo 24, 1661 (1976)
14) Hartiala, K.: Physiol. Rev. 53, 496 (1973)
15) Gibaldi, M. AND PERRIER, D.: Drug Metab. Rev. 3, 185 (1974)
16) Attio, T., Vainio, H. AND Hanninen, O.: FEBS Letter 24, 237 (1972)
17) Hietanen, E.: Pharmacology 12, 84 (1974)
18) SIEKERTS, P.: A. Rev. Physiol. 25, 15 (1963)
19) DALLER, G. AND ERNSTE, L.: J. Histochem. Cytochem. 16, 611 (1968)
20) Op DEN KAMP, J.A.F., Van IJHSTON, W. AND Van Deenen, L.L.M.: Biochim. Biophys. Acta 135, 862 (1967)
21) PapaHadjopoulos, D., Cowden, M. AND Kimelberg, H.: Biochim. Biophys. Acta 230, 8 (1973)
22) Tsai, A.C. AND Dyer, I.A.: J. Nutr. 103, 1119 (1973)
23) BLOCH, K.: Science 150, 19 (1965)
24) Clarke, R.M.: J. Anat. 112, 27 (1972)
25) Marsilos, M., Hanninen, O. AND Hartiala, K.: Nature 213, 918 (1967)
26) TAKESUE, Y. AND SATO, R.: J. Biochem. 64, 873 (1968)