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SHORT COMMUNICATION

Dependence of intestinal biotransformation on dietary cholesterol

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In addition to the liver, extrahepatic tissues are able to metabolize endogenous and foreign compounds by the mixed function oxidases and by conjugating enzymes both in animals and in man [1–12]. Nutritives and numerous xenobiotics enter the body perorally which route provides also a convenient pathway to administer therapeutic drugs. The intestinal microflora and mucosa may convert xenobiotics to metabolically active intermediates capable to induce tumors by binding to cellular macromolecules. On the other hand the mucosa may further metabolize the intermediates or originally reactive compounds into less active form preceding their entrance into the circulation. The mucosal drug metabolism has thus a paramount importance in modifying the biological effects of xenobiotics.

Nutritional factors regulate the activity of hepatic drug metabolizing enzyme system [13–17]. These factors include proteins, carbohydrates, lipids, and trace elements. The diet-induced changes in the lipoidal microenvironment of the hepatic endoplasmic reticulum have been found to regulate hepatic biotransformation rates [18–27]. Much less data exist on the role of diet in the regulation of intestinal drug metabolizing enzymes [6, 12, 19].

In the present study we have compared intestinal biotransformation rates in rats fed cholesterol free diet to those fed 2% cholesterol-enriched diet and conventional pelleted chow. In addition, the compositional changes in the mucosa were detected in an effort to relate compositional variation to changes in enzyme activities.

MATERIALS AND METHODS

Male Wistar rats, purchased as specific pathogen-free and bred in our animals quarters, were used. At the age of 1 month a group of rats was started to be fed cholesterol and fat free pelleted diet (ICN Nutritional Biochemicals Co., Cleveland, OH) and another group was fed conventional pelleted chow containing 0.04% cholesterol and 3.5% other lipids (Hankkija Co-op., Helsinki, Finland). Rats were fed cholesterol free diet for 5 weeks whereafter this group was divided into two subgroups. One subgroup continued to be fed cholesterol free diet but another was fed 2% cholesterol-enriched pelleted diet (ICN Nutritional Biochemicals Co.) for 4 weeks. The protein content of the diets remained constant. At the age of 3 months rats were sacrificed by a blow on the head and bled by cutting the renal vessels. The 10 cm segment of the oral small intestine was dissected and placed in 0.25 M sucrose at 0°. The intestine was opened and cleaned by a moistened piece of paper and the mucosa was scraped off by an ampoule file, weighed and homogenized in four times the wet wt volume of 0.25 M sucrose with a Potter–Elvehjem type glass–Teflon homogenizer (370 rpm for 15 sec). The homogenate was centrifuged at 10,000 *g* for 10 min at 0–4° (Sorvall SS-1). The supernatant fraction was used for further analyses. The protein content was analyzed as described by Layne [28]. Membrane fractions were digested by Na-deoxycholate (5%, Fluka AG, Buchs, Switzerland) before adding the biuret reagent. After the measurement the biuret complex was made colourless by adding 50 mg KCN to each sample to estimate the possible turbidity due to the lipids [29]. The total cholesterol content of the mucosal postmitochondrial supernatant was measured as described earlier [30, 31]. The protein and cholesterol contents were analyzed in frozen samples.

The activity of NADPH cytochrome *c* reductase (E.C. 1.6.2.4) was determined by monitoring the reduction of cytochrome *c* at 550 nm in a Beckman Model 24 spectrophotometer [32]. The assay mixture consisted of 0.1 M Na-phosphate buffer at pH 7.4, 0.3 mM KCN, 0.05 mM cytochrome *c* (Sigma Chemical Co., St. Louis, MO), 0.15 mM NADPH (Sigma) and about 0.6 mg of microsomal protein in postmitochondrial supernatant. The aryl hydrocarbon hydroxylase (E.C. 1.14.14.2) activity was measured using 3,4-benzpyrene (Sigma) as a substrate (0.16 mM added in 25 μl of ethanol) in the presence of 50 mM Tris–HCl buffer pH 7.4, 1 mM $NADP^+$ (Sigma), 5 mM $MgCl_2$ 0.005 mM $MnCl_2$, 2.5 mM Na_3-isocitrate (Fluka) and 0.5 IU pig heart isocitrate dehydrogenase (Boehringer, Mannheim, FRG) and postmitochondrial supernatant equivalent to 0.6–0.8 mg protein in a final volume of 0.5 ml. After a 20 min incubation at 37° the reaction was stopped by 1 ml of cold acetone and the amount of hydroxylated 3,4-benzpyrene was measured by a Perkin–Elmer MPF-2A fluorometer as described earlier [33, 34]. The UDPglucuronosyltransferase (E.C. 2.4.1.17) activity was measured using a uridine diphosphoglucuronic acid (ammonium salt, Sigma) concentration of 4.5 mM in 0.5 M phosphate buffer, pH 7.0 and 0.35 mM *p*-nitrophenol (E. Merck AG, Darmstadt, FRG) as an aglycone [35]. The enzyme assays were made with the fresh tissue. Student's '*t*'-test was used to evaluate statistical significance of the data. Rats fed cholesterol free diet were used as a reference group.

RESULTS AND DISCUSSION

The body wt were about 10 per cent ($2P < 0.05$) higher in rats fed 2% cholesterol or conventional diet in comparison with those rats fed cholesterol free diet (Table 1). No significant differences were found in the mucosal wet wt between various groups. The mucosal postmitochondrial supernatant protein content was nearly significantly elevated in rats fed cholesterol diet (Table 1). The mucosal cholesterol contents were highest in rats fed cholesterol-rich diet, and also in rats fed normal chow the cholesterol content was significantly higher than in the mucosa of rats fed cholesterol free pellets (Table 1). The intestinal cholesterol content was highly dependent on the dietary cholesterol intake. However, even in the mucosa of rats fed normal pelleted diet containing only 0.04% cholesterol the cholesterol content was significantly higher than in the rats deprived totally from dietary cholesterol. In this group the diet contained also neutral lipids possibly facilitating the utilization of dietary cholesterol. In earlier studies the total hepatic cholesterol content has also been shown to be dependent on the dietary cholesterol [20, 25, 27]. Hep-

Table 1. The body weights, mucosal wet weights and protein and cholesterol contents of rats fed cholesterol free, 2% cholesterol and normal pelleted diets

Diet	Body wt (g)	Mucosal wt (10 cm) (g)	Protein (mg/g)	Cholesterol (μmoles/g)
Cholesterol free	276 ± 15	0.60 ± 0.06	80.1 ± 2.7	1.20 ± 0.05
2% Cholesterol	318 ± 6*	0.47 ± 0.04	91.0 ± 2.2*	2.04 ± 0.04‡
Normal	318 ± 10*	0.50 ± 0.03	89.6 ± 3.5	1.50 ± 0.03‡

The mucosal protein and cholesterol contents were measured in postmitochondrial supernatant. The number of rats was five in each group and means and SEMs are given. The statistical significance is expressed by asterisks using cholesterol free group as a reference as follows:
* $2P < 0.05$; † $2P < 0.01$; ‡ $2P < 0.001$.

atic cholesterol synthesis is depressed by the dietary cholesterol which thus increases exogenous proportion of microsomal cholesterol [36]. Harry *et al.* [36] found that the increase in the microsomal cholesterol content was mainly due to the increase in the amount of cholesterol esters while the content of free cholesterol remained constant.

Although lipids are known to be essential for the function and induction of mixed function oxidase enzymes in the liver, much less is known about the response of intestinal biotransformation enzymes to dietary manipulations [22]. The existing data suggest that intestinal mixed function oxidases and UDPglucuronosyltransferase behave differently to dietary fat deficiencies [19]. The NADPH cytochrome *c* reductase activity was not enhanced by cholesterol feeding (Fig. 1a). In the mucosa of rats fed normal pelleted chow the activity was significantly higher than in rats fed cholesterol free or cholesterol supplemented diets. This may be due to the fact that standard diet, on the contrary to cholesterol rich and cholesterol free diets, contains 3.5% other lipids. These neutral lipids may provide additional components needed for the activation of NADPH cytochrome *c* reductase.

The intestinal aryl hydrocarbon hydroxylase activity was enhanced 70-fold by dietary cholesterol on protein basis and also in rats fed normal diet the activity was much higher than in the cholesterol free group (Fig. 1b). In the mucosa of rats fed cholesterol free diet the activity was barely detectable. In recent studies it has been suggested that polycyclic hydrocarbons in trace amounts in the diet might contribute to maintain intestinal monooxygenase activity [7, 10, 12, 37]. Our data support that also a natural component, cholesterol, in the diet is essential in maintaining the intestinal hydroxylative activity.

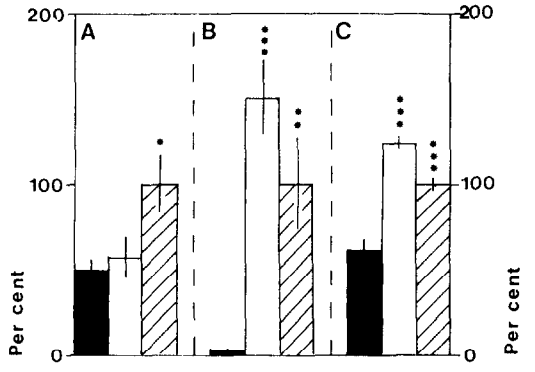

Fig. 1. The NADPH cytochrome *c* reductase (a), aryl hydrocarbon hydroxylase (b) and UDPglucuronosyltransferase (c) activities in the duodenal mucosa of rats fed cholesterol free (black column), 2% cholesterol (white column) and normal (cross-hatched column) diets. The activities are expressed as per cent of the respective activity in the mucosa of rats fed normal pellets. The actual activities per mg of protein in the normal group were following: NADPH cytochrome *c* reductase 26 ± 4 nmoles cytochrome *c*/min, aryl hydrocarbon hydroxylase 4.1 ± 1.1 pmoles of hydroxylated benzpyrene/min and UDPglucuronosyltransferase 0.93 ± 0.04 nmoles *p*-nitrophenol conjugated/min.

The UDPglucuronosyltransferase activity was doubled by dietary cholesterol (Fig. 1c). In the mucosa of rats fed normal pellets the activity was also significantly higher than in rats fed cholesterol free pellets. However, the cholesterol diet increased the UDPglucuronosyltransferase activity more than normal diet ($2P < 0.01$). The enhancement of aryl hydrocarbon hydroxylase and UDPglucuronosyltransferase activities by dietary cholesterol in our study suggests the dependence of intestinal biotransformation reactions on dietary cholesterol. It is possible that cholesterol as an integral part of the endoplasmic reticulum regulates the physicochemical characteristics of the membranes and the membrane fluidity [18, 26]. As the biotransformation enzymes are strictly bound to the endoplasmic membranes and cholesterol regulates membrane properties, it is well possible that changes in the membrane cholesterol level are reflected in the activities of mucosal enzymes.

In addition to enzyme inducers the normal dietary components are evidently able to activate intestinal biotransformation enzymes. In the present study we demonstrated the enhancement of aryl hydrocarbon hydroxylase and UDPglucuronosyltransferase activities in the intestinal mucosa by dietary cholesterol.

SUMMARY

The 2% cholesterol feeding for 4 weeks elevated intestinal cholesterol contents from 1.20 ± 0.05 μmoles/g in rats fed cholesterol free diet to 2.04 ± 0.04 μmoles/g. The mucosal aryl hydrocarbon hydroxylase activity was enhanced from 0.086 ± 0.043 pmoles/min × mg (protein) to 6.11 ± 0.89 in cholesterol fed rats and to 4.07 ± 1.08 found in rats fed normal pellets. No increase was found in the mucosal NADPH cytochrome *c* reductase activity by cholesterol diet although normal pellets increased the activity from that found in the cholesterol free group. The UDPglucuronosyltransferase activity was doubled by dietary cholesterol. The data suggest that dietary cholesterol enhances intestinal hydroxylation and glucuronidation rates. This increase may be mediated by the compositional changes in the microenvironment of the membrane-bound enzymes.

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