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Biochemical Pharmacology, Vol. 25, pp. 2750–2752. Pergamon Press, 1976. Printed in Great Britain.

Response of microperoxisomes in rat small intestinal mucosa to CPIB, a hypolipidemic drug

(*Received* 19 *January* 1976; *accepted* 26 *March* 1976)

Since their identification and chemical characterization, peroxisomes (microbodies) of rat liver have been studied rather extensively [1–4], while those in other organs and tissues have received relatively less attention.

Peroxisomes were first discovered in rodent liver and kidney; subsequently they have been reported in a wide variety of tissues of vertebrates [5, 6] as well as in plants. Recently, Novikoff and Novikoff [7] reported large numbers of peroxisomes in the duodenum, jejunum and ileum of guinea pigs and in rat duodenum. These authors pointed out that peroxisomes of intestinal absorptive cells lacked nucleoids, were of a smaller diameter than those of liver or kidney, and had numerous slender continuities with smooth endoplasmic reticulum membrane. Accordingly, these authors proposed the term "microperoxisome" for this smaller population of organelles. Connock and Kirk [8], using isopycnic centrifugation of homogenates of guinea pig intestinal epithelium, separated a fraction rich in catalase particles that sedimented more slowly than lysosomes and mitochondria and which are probably identical to intestinal microperoxisomes as described in this tissue by the Novikoffs. Pipan and Psenicnik [9] identified microperoxisomes in small intestinal epithelium of 13- to 19-day mouse embryos and found that their number increased with increasing age of the animal and differentiation of the cells in which they were found.

In the present paper, the terms "peroxisome" and "microperoxisome" are used interchangeably and both are regarded as closely related to the term "microbody" as used in earlier literature as a purely morphological term describing hepatic or renal peroxisomes.

It had been shown previously that, in male rat liver, peroxisomes increased in number and one of their principal enzymes, catalase, increased in activity after administration of ethyl alpha-chlorophenoxyisobutyrate (CPIB), a hypolipidemic drug. The results of a number of other studies suggested that peroxisomes may be related to lipid and steroid metabolism [10–15]. Because of the possible relationship of peroxisomes to lipid metabolism and in view of the fact that the intestinal tract may be the major site of cholesterol synthesis in the rat [16, 17], it was desirable to study the response of intestinal peroxisomes to a hypolipidemic agent known to cause significant alterations in lipid metabolism and in hepatic peroxisomes and their enzymes. Moreover, Novikoff and Novikoff [7] suggested that, because of the abundance, size and structure of peroxisomes in intestinal epithelium, they might be favorable for biochemical study. Accordingly, the present study was undertaken as a preliminary step to compare intestinal peroxisomes to those in the rat liver with regard to their response to CPIB.

Materials and methods

F-344 rats weighing 150–200 g were caged individually and given water *ad lib.* Fifteen males and ten females were fed 0.25% CPIB in their diet for 3 weeks; ten rats of each sex served as controls. Animals were fasted overnight and sacrificed between 9:00 and 10:00 a.m. The livers were removed, weighed and samples taken for cytochemical and biochemical determinations. For samples of intestine, the first 10 cm distal to the pyloric sphincter were omitted; the next 20-cm segment of small intestine was removed and designated jejunum. The 20-cm segment of small intestine proximal to the ileocecal valve was removed and designated ileum. Both segments of small intestine were opened longtitudinally and the lumen was flushed rapidly with cold saline. The segments were then placed serosa side downward on parafilm and the mucosa and submucosa were separated from muscularis by scraping gently with the edge of a glass slide. To insure the efficacy of this method for separation of mucosa from remaining intestinal wall, microscopic sections were prepared from the mucosal scrapings and from the remaining tissue.

Biochemical and cytochemical methods. One-g samples of liver and intestinal mucosa were homogenized in 4 ml of M/150 phosphate buffer at 1–4° in a Potter–Elvehjem homogenizer. Catalase activity was measured by the spectrophotometric method of Lück [18]. Total proteins were determined on liver and intestine by the method of Lowry [19]. For cytochemical demonstration of peroxidatic activity of catalase, small portions of liver and intestine were fixed in 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate, pH 7.4, for 4 hr at 4°. Tissues were rinsed overnight in 0.1 M cacodylate buffer containing 0.2 M sucrose. Slices of minced tissue were incubated at 37° for 45–60 min in the 3,3′-diaminobenzidine medium of Novikoff and Goldfischer [20, 21] modified from Graham and Karnovsky [22]. The incubation medium contained 10 mg of 3,3′-diaminobenzidine tetrahydrochloride (DAB, Sigma Chemical Co., St. Louis, Mo.) in 10 ml of 0.05 M 2-amino-2-methyl-1,3-propanediol buffer, pH 9.4, and 0.2 ml of 1% hydrogen peroxide. Controls consisted of: (1)

Table 1. Catalase activity in liver, jejunum and ileum of F-344 rats of both sexes who received 0.25% CPIB (clofibrate) in ground chow diet for 3 weeks

Group	No. of rats	Catalase activity (units catalase/mg protein)		
		Liver	Jejunum	Ileum
Male controls	10	46.01 ± 1.3	0.47 ± 0.07	0.59 ± 0.07
Males CPIB	15	74.23 ± 3.6	0.89 ± 0.05	1.13 ± 0.05
Student's *t*-value		7.47	4.21	6.35
Per cent increase over controls		61	82	92
Significance		>0.001	>0.001	>0.001
Female controls	10	35.5 ± 1.3	0.70 ± 0.02	0.73 ± 0.03
Female CPIB	10	42.98 ± 2.3	0.85 ± 0.05	0.75 ± 0.05
Student's *t*-value		3.56	3.06	0.33
Per cent increase over controls		21	21	2.7
Significance		>0.005 <0.001	>0.005 <0.001	>0.70 <0.60

preincubation for 15 min in propanediol buffer containing 0.02 M 3-amino-1,2,4-triazole followed by incubation in standard DAB medium also containing 0.02 M aminotriazole, (2) perincubation for 10 min in propanediol buffer containing 0.1 M KCN followed by incubation in standard DAB incubation mixture also containing 0.1 M KCN, and (3) incubation in the standard DAB incubation mixture lacking hydrogen peroxide.

Results

The biochemical results are given in Table 1. Consistent with previous findings [23, 24] in liver, the increase in catalase activity after CPIB was greater in males (61 per cent increase over control value) than in females (21 per cent over control value). Similarly, in the mucosa of jejunum and ileum of male rats, CPIB elicited an increased catalase activity over control value of 82 and 92 per cent respectively. In jejunum and ileum of female rats, on the other hand, the catalase activity increased only 21 and 2.7 per cent, respectively, over the controls. The increase in ileum is not significant.

The morphological studies, for the most part, confirmed the biochemical findings. With routine electron microscopic methods, only an occasional structure compatible with a microperoxisone was apparent in control cells. In males given CPIB, the number of such organelles was increased and the increase was apparent by visual inspection of representative micrographs of jejunum and ileum as well as of liver. Similarly, with DAB incubation, the number of microperoxisomes, indicated by reaction product, was greater in CPIB-fed males than in controls. In females, no increase in microperoxisomes could be discerned in sections of liver, jejunum or ileum.

Discussion

As pointed out by Novikoff and Novikoff [7] intestinal peroxisomes are generally smaller than those of liver and they lack interior crystalloids or nucleoids that are typical of hepatic peroxisomes of several species. Accordingly, the Novikoffs proposed the term "microperoxisome" for the small peroxisomes of intestinal absorptive epithelial cells. While conceding the morphological differences in peroxisomes as emphasized by Novikoff, it seems reasonable to regard both hepatic and intestinal peroxisomes as belonging to the same class of cell organelles and, consequently, it is logical to compare the biological behavior of the organelles in different tissues despite apparently minor differences in their morphology.

A number of studies have suggested that peroxisomes are related to lipid [11, 25] metabolism or to the synthesis, storage or utilization of cholesterol [12, 13, 26]. In this regard, it is noteworthy that the intestinal mucosa not only plays a role in lipid transport but recently it has been shown that, in rats, between 55 and 56 per cent of cholesterol synthesis takes place in the intestine [16, 17]. If microperoxisomes are indeed involved in cholesterol metabolism, their presence in large numbers in intestinal mucosa of rats given CPIB may be related to alterations in intestinal cholesterol synthesis or transport induced by this hypolipidemic drug.

It is noteworthy that the response of intestinal mucosal catalase activity is similar to hepatic catalase activity in male rats fed CPIB. Moreover, just as increased hepatic catalase activity and peroxisome proliferation in response to CPIB are greater in males than in females, the same is true for these responses in the intestinal mucosa. At present, the mechanisms that govern hepatic peroxisome proliferation in response to CPIB and other hypolipidemic drugs are not known but it would appear that, whatever the mechanism, a similar one influences intestinal mucosal microperoxisomes. In any case, some aspect of cholesterol metabolism may be common to both male liver and intestinal mucosa accounting for the similarity of catalase/peroxisome response in these two tissues.

In the liver, proliferation of peroxisomes and increased catalase activity are independent of thyroxin and adrenal hormones but require androgenic hormones [23]. It remains to be determined whether such endocrine factors similarly influence intestinal mucosal microperoxisomes.

In view of the distribution of peroxisomes in a wide variety of vertebrate tissues and cell types, it will be of interest to determine whether, in addition to intestinal mucosal peroxisomes, those of other tissues respond to CPIB or other more potent hypolipidemic agents. If responses of peroxisomes in different tissues are dissimilar in response to hypolipidemia or to endocrine hormones,

for example, it would support the suggestion of Hruban *et al.* [6] that peroxisomes probably have a variety of functions in vertebrates depending on the tissue in which they occur.

In summary, feeding 0.25% CPIB, a hypolipidemic drug, caused significant increase in catalase activity and in the number of microperoxisomes in the intestinal mucosa of male rats. This increased catalase activity may be related to the important role of intestinal mucosa in cholesterol synthesis.

Acknowledgement—These studies were supported in part by United States Public Health Service Grant CA-5680.

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Biochemical Pharmacology, Vol. 25, pp. 2752–2754. Pergamon Press, 1976. Printed in Great Britain.

Dopamine—adaptive uptake changes in striatal synaptosomes after 30 sec of shock-induced fighting

(*Received* 15 *March* 1976; *accepted* 12 *May* 1976)

It has been found recently that adaptive kinetic changes in the neuronal uptake of norepinephrine (NE) take place in response to various forms of stress. These include electro-convulsive shock similar to that used in the treatment of depression [1, 2], acute head trauma,* and fighting [2–4].

In the latter instance, isolated mice subjected to chronic, daily fighting episodes exhibit an increased maximum velocity (V_{max}) for NE uptake in synaptosome-rich homogenates of cerebral cortex [3]. At the same time, there is a decreased carrier receptor affinity for this catecholamine, as indicated by an increased value for the Michaelis constant (K_m). When apparent Michaelis–Menten kinetics for NE uptake are also applied to acutely fighting isolated mice [2], there is little if any increase in V_{max}, but again the K_m is increased, a change which reverses itself after 24 hr. However, both V_{max} and K_m for NE uptake are strikingly increased in synaptosomes isolated from retired male breeding mice that fight vigorously for only 5 min [4].

In the present study, we investigated dopamine (DA) uptake to see whether this, too, was altered by fighting. We used a more intense fighting model than those previously referred to and we measured dopamine uptake in striatal synaptosomes after just 30 sec of combat to see if kinetic changes like those described above occurred almost immediately. Finally, we tested the effects of diphenylhydantoin (DPH, Dilantin) on DA uptake in our fighting animals because it has already been shown that DPH inhibits the enhanced uptake of NE induced by fight-

* W. Adams, M. G. Hadfield, W. F. C. Rigby and D. P. Becker, manuscript in preparation.