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Expression and localization of cysteine dioxygenase mRNA in the liver, lung, and kidney of the rat

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Summary. The expressions of cysteine dioxygenase (CDO) gene in the liver, lung, skeletal muscle, and kidney were studied by *in situ* hybridization with a cDNA probe from rat liver CDO under normal conditions. Significant expression of the CDO gene was detected in the liver, lung, and kidney, but not skeletal muscle. In the liver, the signal was confined to the cytoplasm of the hepatocytes. Furthermore, the signal was stronger in the periportal than that in the perivenous areas. In the lung, an intensive signal was found in the bronchiolar epithelium. As to the kidney, an intensive signal was observed in the distal convoluted tubules, while no signal was found in the proximal convultions.

Keywords: Amino acids – *In situ* hybridization – Cysteine dioxygenase – Liver – Lung – Kidney – Rat

Introduction

Cysteine dioxygenase (CDO; EC 1.13.11.20) catalyzes the oxygenation of the thiol group of L-cysteine to yield L-cysteinesulfinate, which has a key role for taurine and cysteinesulfinate synthesis in mammals. This enzyme was purified from rat liver, and its properties were previously elucidated (Sakakibara et al., 1973, 1976; Yamaguchi et al., 1978). We have isolated a cDNA from rat liver CDO and characterized it (Hosokawa et al., 1990). The cDNA, designated rCDO-39, contained a insert of 1,458 bp and encoded a polypeptide of 23,025 Da consisting of 200 amino acids (Hosokawa et al., 1990; Tsuboyama et al., 1996).

In the rat liver, which synthesizes a large amount of taurine, CDO plays a major role in the regulation of cysteine catabolism, especially in the conversion of cysteine to taurine (Yamaguchi et al., 1973). It has been reported that the hepatic CDO activity of rats is elevated by injection of glucocorticoid, and

is also elevated by either high protein or high sulfur amino acid intake (Yamaguchi et al., 1973). Taurine, on the other hand, is produced from cysteine much faster in perivenous areas than in periportal rat hepatocytes (Penttilä, 1990). During incubation with methionine, the taurine content of perivenous hepatocytes was somewhat higher and increased faster than that of periportal areas (Pentillä, 1990). Zonal differences have also been noted for cysteine uptake in rat liver. In the perivenous zone of the hepatic acinus, a low affinity, high capacity transport system for cysteine is localized (Saiki et al., 1992). Therefore, the metabolism of taurine is highly zonated within the acinus (Pentillä, 1990) as are other amino acids and carbohydrates (Häussinger et al., 1992; Jungermann and Thurman, 1992). Furthermore, relevant to certain amino acids and carbohydrates, zonation of gene expression of several enzymes has also been reported. The present study, firstly, focused on whether these zonal gene expressions are also present in taurine metabolism, and was designed to see the zonation of the CDO mRNA in rat liver lobule by *in situ* hybridization. At the same time, the location of the CDO mRNA was also observed in the lung and kidney, because, in the previous study the gene expression of CDO was detected by Northern blot analysis (Hosokawa, 1990; Tsuboyama, 1996).

Materials and methods

Animals and tissues

Five week-old male Sprague-Dawly rats weighing 140–150 g were maintained with laboratory chow for a week. The animals were anesthetized by intraperitoneal injection with pentobarbital (40 mg per kg body weight), and perfused transcardically with 4% paraformaldehyde in 0.1 M phosphate buffer of pH 7.4. After perfusion, liver, lung, kidney, and skeletal muscle (the soleus muscle) were removed, fixed in 4% buffered paraformaldehyde, and embedded in paraffin by routine procedures. Five mm thick sections were mounted on 3-aminopropyltriethoxysilane coated slides. The slides were then heated at 60°C for 2 hr, and stored at 4°C until ready for use.

Preparation of RNA probe

A 1.4 kb fragment, containing coding sequences for rat CDO, generated from rCDO-39 by PCR was inserted into insertion sites of pGEM −7Zf(−) vector (Promega Co., WI, USA) for *in vitro* transcription. Anti-sense RNA probes were synthesized using SP6 polymerase after linearization of the plasmid with Nco I. Sense strand RNA probes were synthesized using T7 polymerase after linearization of the vector with Spe I. The probes were synthesized using a digoxygenin-RNA labeling kit (Boehringer-Mannheim, Germany).

In situ hybridization

Deparaffinized sections were treated with proteinase K, acetylated, dehydrated with graded ethanol solutions, and air dried. They were hybridized for 16 hr at 50°C in a hybridization buffer (50% deionized formamide, 10 mM Tris-HCl pH 7.6, 200 μg/ml tRNA, 1 × Denhardt's solution, 10% dextran sulfate, 600 mM NaCl, 0.25% SDS, 1 mM EDTA pH 8.0) containing the labeled RNA probe. After hybridization the sections were washed with 50% formamide / 2 × SSC (twice at 50°C for 30 min), treated with RNase

(10 mg/ml in 10 mM Tris-HCl pH 8.0, 0.5 M NaCl, 1 mM EDTA pH 8.0) at 37°C for 20 min, washed in 50% formamide / 2 × SSC (twice at 50°C for 30 min), 50% formamide / 1 × SSC (twice at 50°C for 30 min), 1 × SSC (at 50°C for 20 min) and 0.2 × SSC (twice at 50°C for 20 min). Digoxygenin detection was carried out using a digoxygenin detection kit (Boehringer-Mannheim, Germany). Safranin O was used as a counterstain.

Results

To observe the localization of mRNA of rCDO, we performed *in situ* hybridization studies on tissue sections of rat liver, lung, kidney, and skeletal muscle (the soleus muscle). The hybridization probe was a cRNA generated from a 1.4 kbp rat liver cDNA fragment. In the liver, with digoxygenin-labeled antisense cRNA CDO probes, strong, dark, and finely granular staining were limited in the cytoplasm of hepatocytes and were not found in the endothelial cells (Fig. 1b). Positive staining was confined to the cytoplasm, which sharply outlined the clear unstained nuclei. No hybridization signals were detectable in control experiments using a digoxygenin-labeled sense cRNA CDO probe (Fig. 1a). Significant differences in staining intensity were observed between the periportal and perivenous area. The signal was stronger in the periportal area than in the pericentral venous area (Fig. 1c). In contrast to the periportal area, the perivenous area showed only a minimal signal.

In the lung, an intensive signal was detected in the cytoplasm of the bronchiolar epithelium, when the antisense cRNA CDO probe was used (Fig. 2a). In the kidney, the intensive signal was seen in the distal convoluted tubule, while no signal was detected in the proximal convolution (Fig. 2b). The signals were detected in the cytoplasm of the epithelium of the distal tubules. Figure 2c illustrates that the signal was absent on a section of soleus muscle of antisense cRNA CDO probe.

Discussion

In the present study, mRNA of rCDO in the liver, lung, kidney and skeletal muscle was investigated by means of *in situ* hybridization techniques. It is obvious that specific CDO transcripts occur in the liver, lung, and kidneys, but not in the skeletal muscle. These results were in good agreement with Northern blot analysis (Tsuboyama et al., 1966). In the previous work, the analysis was carried out with rat CDO cDNA (rCDO-39), and demonstrated that a strong hybridization band of CDO mRNA was detected in the liver RNA with approximate size of 1.7 kb, while no detectable mRNA was found in RNA isolated from skeletal muscle (Tsuboyama et al., 1966). Significant levels were also observed in the kidney, lung and brain (Tsuboyama et al., 1966).

A unique feature of hepatic physiology is the heterogeneous distribution of function of the hepatocytes (Rappaport 1960). From the view point of the blood stream, at least three different zones can be arbitrarily differentiated: the periportal area (afferent zone I of Rappaport) which is supplied by high oxygen and specific substrates (e.g. bile acids); the perivenous area (efferent

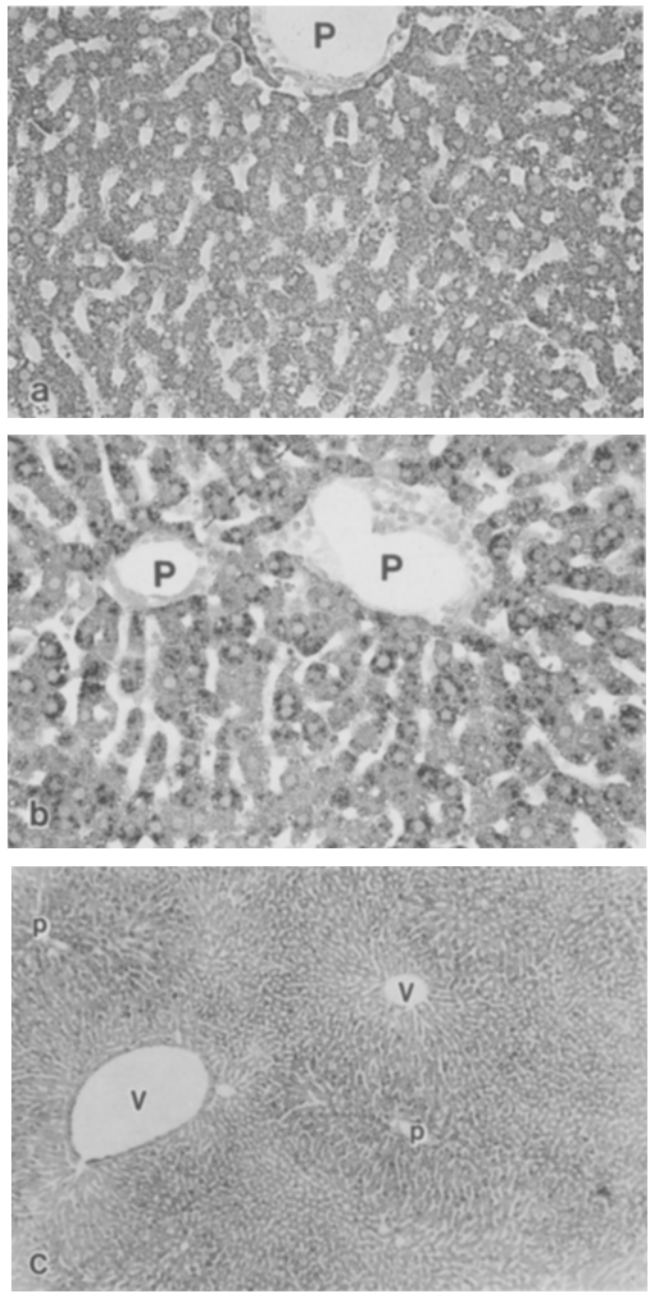

Fig. 1a–c. **a** High-power microphotograph of the rat liver after *in situ* hybridization using sense cRNA CDO probe (10 × 40). No signal is seen in the liver. *P* portal vein. **b** Microphotograph of the rat liver after *in situ* hybridization using antisense cRNA CDO probe (10 × 40). Significant expression of the CDO gene was detected in the cytoplasm of hepatocytes. *P* portal veins. **c** Low-power photograph of the rat liver *in situ* hybridization using antisense cRNA CDO probe (10 × 10). The signal is stronger in the periportal area than in the pericentral venous area. *V* central veins, *P* portal veins

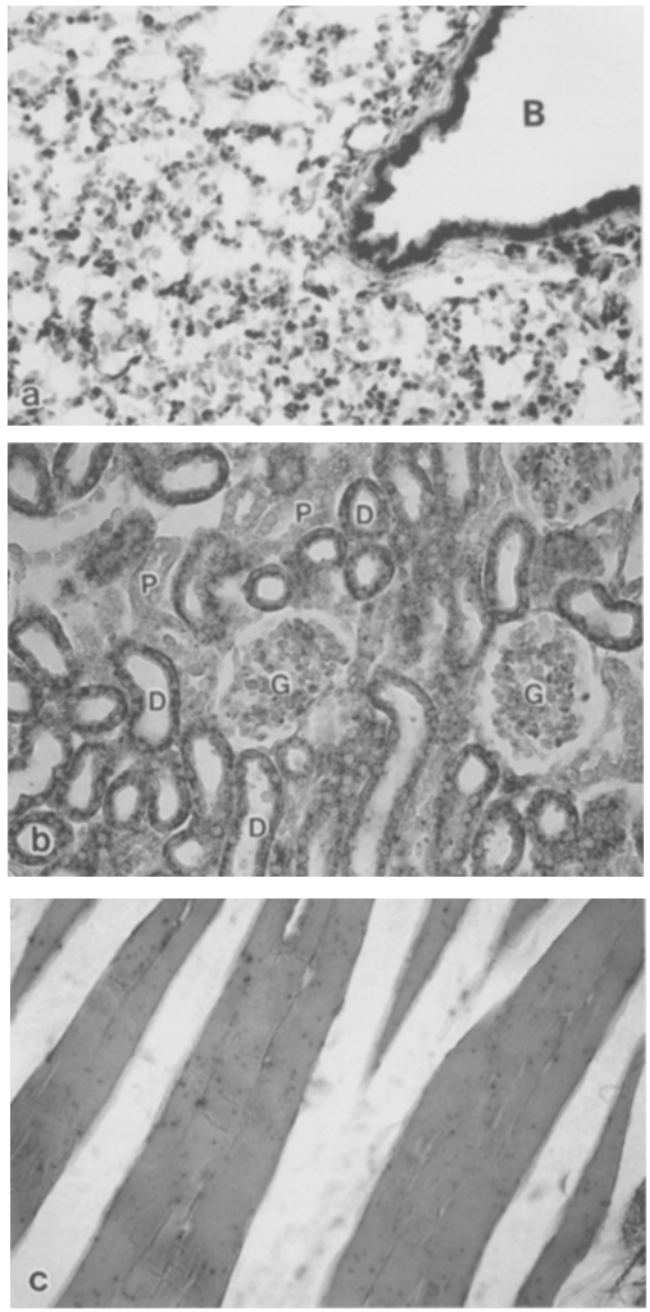

Fig. 2a–c. **a** Microphotograph of the rat lung after *in situ* hybridization using antisense cRNA CDO probe (10 × 40). Intensive signals are detected in the cytoplasm of the bronchiolar epithelium. *B* bronchiole. **b** High-power microphotograph of the rat kidney after *in situ* hybridization using antisense cRNA CDO probe (10 × 40). Intensive signals are observed in the distal convoluted tubule (*D*), while no signal was detected in the proximal convolution (*P*). The distal convoluted tubule has such characteristic features as the absence of a brush border and a larger, and more clear lumen. Signals were detected in the cytoplasm of the epithelium of the distal tubule. *G* glomerulus. **c** Microphotograph of the rat skeletal muscle (soleus muscle) after *in situ* hybridization using anti-sense cRNA CDO probe (10 × 40). Skeletal muscle shows no signal

zone or zone III of Rapaport) which receives low oxygen and specific substrates; and the intermediate area between them which is normal zone II or the midzone. Since the composition of metabolites in the blood changes during flow through sinusoids, cells in the different zones of the liver parenchyma might possess different structural and enzymatic composition and function. In fact, it has been established that a number of enzymes involved in various metabolic activities are unequally distributed within the hepatocytes (Häussinger et al., 1992; Jungerman and Thurman, 1992; Traber et al., 1988). In the liver, zonal differences of CDO mRNA were also found. Transcripts of CDO mainly occurred in hepatocytes around the portal vein as shown in Fig. 1c. Taurine is synthesized essentially via the formation and decarboxylation of cysteinesulfinate (Rosa and Stipanuk, 1985). The conversion of cysteine to taurine is regulated by the activities of the two enzymes; CDO and cysteinesulfinate decarboxylase (Rosa and Stipanuk, 1985; Weinstein and Griffith, 1987). The observation that the ^{35}S labeled cysteine is incorporated into taurine much faster in perivenous than in periportal hepatocytes of male rat indicates that the capacity for taurine production is strongly zonated in the hepatic acinus (Pentillä, 1990). It has been reported that the basal level of taurine tended to be higher, and to be elevated by methionine during incubation to a greater extent, in perivenous than in periportal hepatocytes (Pentillä, 1990). The present study showed that the periportal hepatic cells contained higher content of the CDO gene than that of perivenous hepatic cells. As stated above, cysteine is converted into cysteinesulfinate by CDO, and cysteinesulfinate is metabolized into hypotaurine by cysteinesulfinate decarboxylase. Taurine synthesis is regulated by these two steps (Rosa and Stipanuk, 1985; Weinstein and Griffith, 1987). These results imply that the cysteinesulfinate produced in periportal hepatocytes might be transported to the perivenous cells via the systemic circulation, where it is converted to taurine via hypotaurine. To fully understand the synthesis of taurine among perivenous cells and periportal cells, it will be necessary to elucidate the zonation of CDO and the cysteinesulfinate decarboxylase protein, and observe the incorporation of cysteinesulfinate into the perivenous cells.

In the lung, CDO mRNA expressed in the bronchiolar epithelium. In addition, taurine can protect lung epithelial cell by converting HOCl/OCl− to anionic monochloramines (Cantin, 1994). Taurine protects rat bronchioles from acute ozone-induced lung inflammation and hyperplasia (Schuller-Levis et al., 1995). So the taurine that is synthesized and secreted from the bronchiolar epithelium may act as a detoxifying antioxidant.

In the kidney, the intensive signal was seen in the distal convoluted tubule, while no signal was detected in the proximal convolution. It is a well-known observation that such electrolytes as Na^+, K^+, HCO_3^-, Cl^-, SO_4^{2-} and PO_4^{3-} are reabsorbed from the proximal tubule. At the same time, a lot of water is also reabsorbed passively from the proximal tubule. Therefore, the proximal tubule acts to isotoniccally reabsorb various metabolites. On the other hand, water reabsorption from the distal tubule is controlled by the antidiuretic hormone. Thus osmotic stress exists in the epithelium of the distal tubule. According to Jones et al. (1992, 1993), taurine transport is greatest at the

apical surface of the cell in the proximal tubule, while it is greatest at the basolateral surface of the cell in the distal tubule. Consequently, the taurine in the epithelium of the proximal tubule is mainly reabsorbed from the urinary tubules. By contrast, the taurine in the epithelium of the distal tubule is synthesized and transported from the basolateral surface. Consequently, taurine in the distal tubule may form a mechanism of cell volume regulation in response to osmotic stress.

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