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Catechol-O-Methyltransferase Activity in Cholestatic Rat's Liver Induced by Bile Duct Ligation

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Abstract: To investigate the cause of increased plasma catecholamine levels in liver disease, catechol-O-methyltransferase (COMT), which provides a major route of catabolism for circulating catecholamines, was studied under the cholestasis induced by mechanical biliary obstruction in rats. Monoamine oxidase (MAO) activity and the K_m and V_{max} values for both enzymes were also measured. Cytosolic, microsomal, and mitochondrial COMT activities in the cholestatic liver were significantly decreased throughout the experiment. Microsomal, and mitochondrial MAO activity in the cholestatic liver were also significantly decreased. Vmax values of COMT and MAO were lower. Serum COMT and MAO activities were detected after CBD ligation. These results indicate that plasma catecholamine levels are increased in liver disease due to decreased hepatic degradation of catecholamines by decreased activities of COMT and MAO. The decreased activity of these enzymes is caused by decreased biosynthesis and by flowage into the blood from the damaged hepatocyte.

Key words: catechol-O-methyltransferase, cholestasis, monoamine oxidase.

Many neurotransmitters and hormones are derivatives of catechol (Granner, 1993). These catecholamines include such important compounds as noradrenalín, adrenalin and dopamine (Granner, 1993). Their function as signalling molecules results in high activities at low concentrations (Granner, 1993). As a result they are usually the first molecules to be affected by a disease, and a study of these signals or of their metabolic pathways can yield important insights about a particular disease.

Catecholamines are metabolized by catechol-O-methyltransferase (S-adenosyl-L-methionine:catechol O-methyltransferase, EC 2.1.1.6; COMT) and monoamine oxidase (EC 1.4.3.4 MAO) (Granner, 1993). Most catecholamines are substrates for both of these enzymes (Granner, 1993). COMT catalyzes the transfer of the methyl group of S-adenosyl-L-methionine to the hydroxyl group of catechols and catecholamines in the presence of magnesium(Tilgmann and Kalkkinen, 1990). The highest level of COMT activity is generally found in the liver (Borchardt and Cheng, 1978; Tilgmann and Kalkkinen, 1990). MAO is an enzyme that catalyzes the oxidative deamination of naturally occurring monoamines (Granner, 1993). It is localized in mitochondria and microsomes (Ghosh and Guha, 1977; Baldessarini, 1991), and occurs in highest concentrations in the liver (Baldessarini, 1991; Granner, 1993). MAO is also important in regulating the metabolic degradation of catecholamines (Baldessarini, 1991). At least 2 isozymes have been described. MAO-A deaminates serotonin and epinephrine, while MAO-B is most active against 2-phenylethylamine and benzylamine (Granner, 1993).

There are many studies of catecholamines in hepatic diseases like cirrhosis (Greco *et al.*, 1985; Gaudin *et al.*, 1990; Gaudin *et al.*, 1991; Floras *et al.*, 1991; Hsu, 1992). It is known that levels of plasma catecholamines are high in patients with cirrhosis (Greco *et al.*, 1985; Gaudin *et al.*, 1990; Gaudin *et al.*, 1991; Floras *et al.*, 1991) and that induction of liver cirrhosis by carbon tetrachloride is promoted in rats with high plasma catecholamine concentrations (Hsu, 1992).

However, in spite of many reports about the increased levels of catecholamines in hepatic cirrhosis, the hepatic COMT which provides a major route of catabolism for circulating catecholamines (Borchardt and Cheng, 1978; Raxworthy and Gulliver, 1986; Tilgmann and Kalkkinen, 1990; Bertocci *et al.*, 1991) has not been studied in liver disease.

To investigate the causes of these increased levels of plasma catecholamine in liver disease, COMT and

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MAO activities were studied under the cholestasis induced by mechanical biliary obstruction in rats. The K_m and V_{max} values of these enzymes were also measured.

Materials and Methods

Animals

Normal male Sprague-Dawley rats weighing between 320 and 350 g were used. All animals were maintained on a diet of commercial pellets purchased from Sam Yang Food Co., Limited. The common bile duct (CBD) was exposed through a middle line incision. After double ligation the mid point of the CBD was cut. A sham operation was performed in the same way without CBD ligation. The sham operated control rats were sacrificed at the 1st, the 2nd, the 3rd, the 7th, the 14th, the 28th, and the 42nd day after sham operation. The CBD ligated rats were sacrificed at the 1st, the 2nd, the 3rd, the 7th, the 14th, the 28th, and the 42nd day after CBD ligation. Rats were anesthetized with ether for surgery and sacrifice, and were fasted prior to sacrifice.

Chemicals

Benzylamine HCl, 5-hydroxytryptamine HCl, S-adenosyl-L-methionine iodide, DL-dithiothreitol, Triton X-100, p-bis-(o-Methylstyryl) benzene, monoamine oxidase, catechol-O-methyltransferase, and bovine albumin were purchased from Sigma (St. Louis, USA). [Methyl-^{3}H] S-adenosyl-L-methionine was purchased from Du Pont (USA), All other chemicals were of the highest commercially available purity.

Cell fractionation

After rats were anesthetized with ether blood was collected from the abdominal aorta, and the liver was perfused through the portal vein with physiologic saline solution. The liver was rinsed in cold saline solution. The surface was then wiped dry. Cytosol, microsome, and mitochondria were obtained according to the method described by Kwak and Kwak (1986). All procedures were performed at 2 to 4℃.

Enzyme assays

The COMT activity was measured with a spectrophotometer (Varian, Cary 210) according to the method of Borchardt (1981). The hepatic MAO activity was measured according to the method of Nagatsu and Yagi (1966) using 5-hydroxytryptamine as a substrate for MAO-A, and benzylamine for MAO-B. The serum MAO activity was measured according to the method of McEwen and Cohen (1963).

Determination of protein

Protein concentrations were determined by the biuret method using bovine serum albumin as a reference protein.

Statistical analysis

Values were expressed as mean± S.D. Statistical evaluation of the difference between means was performed with Student's t-test.

Results

Cytosolic, microsomal, and mitochondrial COMT activity in the cholestatic liver was significantly decreased between the 1st day and the 42nd day after CBD ligation (Table 1).

V_{max} values of COMT in cytosolic, microsomal, and mitochondrial fractions were decreased after CBD ligation, but K_m values showed no significant change (Table

Table 1. Activity of hepatic catechol O-methyltransferase (COMT) in cholestatic rat liver

Day(s) following ligation	COMT activities (pmol 3-hydroxy-4-methoxybenzoic acid with) 4-hydroxy-3-methoxybenzoic acid $min^{-1} \cdot mg$ protein^{-1})					
	Cytosol		Microsome		Mitochondria	
	Sham	CBDL	Sham	CBDL	Sham	CBDL
1(n=8)	2,898± 486	2,389± 321^a	427± 69	345± 42^a	110± 21	84± 13^a
2(n=8)	2,921± 472	2,251± 312^b	432± 67	341± 48^b	113± 19	83± 14^b
3(n=8)	2,912± 463	2,048± 274^c	426± 71	300± 36^c	112± 22	80± 15^b
7(n=8)	2,896± 468	2,012± 262^c	420± 66	292± 41^c	109± 18	79± 14^b
14(n=5)	2,873± 454	1,972± 244^b	416± 64	279± 39^b	106± 19	76± 12^a
28(n=5)	2,862± 442	1,858± 198^b	417± 65	236± 33^c	108± 16	74± 9^b
42(n=5)	2,854± 446	1,756± 212^b	415± 62	210± 28^c	105± 20	71± 10^b

Values are mean± S.D. Sham: sham operated control rats; CBDL: common bile duct ligated rats.
Values significantly different from sham operated control values ($^a p<0.05$, $^b p<0.01$, $^c p<0.001$).

Table 2. Catachol O-methyltransferase (COMT) kinetic parameters determined with dihydroxybenzoic acid

Cell fractions	K_m (mM)		V_{max} (pmol 3-hydroxy-4-methoxybenzoic acid with 4-hydorxy-3-methoxybenzoic acid $min^{-1}\cdot mg\ protein^{-1}$)	
	Sham	CBDL	Sham	CBDL
Cytosol	2.88±0.69	3.16±0.76	4,495±837	3,044±512[a]
Microsome	4.94±0.97	4.71±1.12	181± 28	128± 21[b]
Mitochondria	3.18±1.02	3.37±1.24	724±141	432± 78[b]

Michaelis-Menten constants for COMT were determined using 3, 4-dihydroxybenzoic acid, S-adenosyl-L-methionine iodide and [methyl-^{3}H]S-adenosyl-L-methionine from cholestatic rat livers at 28th day after CBD ligation. Values are mean± S.D. with 5 rats in each group. Sham : sham operated control rats; CBDL : common bile duct ligated rats. Values significantly different from sham operated control values ([a]$p<0.05$, [b]$p<0.01$).

2).

Serum COMT activity in sham operated rats was not detected, while that in CBD ligated rats was detected (Table 3).

Mitochondrial MAO-A activity in the cholestatic liver was significantly decreased between the 3rd day and the 42nd day after CBD ligation (Table 4), and mitochondrial MAO-B activity was significantly decreased between the 1st day and the 42nd day after CBD ligation (Table 4). Microsomal MAO-A, and MAO-B activities in the cholestatic liver were significantly decreased between the 28th day and the 42nd day after CBD ligation (Table 4).

V_{max} values of MAO-A and MAO-B in mitochondrial and microsomal fractions were decreased after CBD ligation, but K_m values showed no significant change (Table 5).

Serum MAO activity in sham operated rats were not detected, while that in CBD ligated rats were detected between 7th day and 42nd day (Table 6).

Discussion

Mechanical biliary obstruction in rats by CBD ligation causes necrosis and inflammation from 1 day after CBD ligation, fibrosis from 7 or 14 days after CBD ligation, and cirrhotic changes from 28 or 42 days after CBD ligation with the functional abnormality in the liver (Kountouras *et al.*, 1984; Chang *et al.*, 1987; Kim *et al.*, 1989). In this experiment, cytosolic, microsomal, and mitochondrial COMT activities in the cholestatic liver were significantly decreased throughout the experiment. Microsomal and mitochondrial MAO activities in the cholestatic liver were also significantly decreased. These results indicate that hepatic degradation of catecholamines by COMT and MAO is decreased from the inflammatory stage to cirrhotic stage in liver disease and that this results in the increase of circulating catecholamines reported by others (Greco *et al.*, 1985;

Table 3. Activity of serum catechol O-methyl transferase (COMT) in cholestatic rat

COMT activities (pmol 3-hydroxy-4-methoxybenzoic acid with 4-hydroxy-3-methoxybenzoic acid $min^{-1}\cdot mg\ protein^{-1}$)							
Sham	CBDL 1st	2nd	3rd	7th	14th	28th	42nd day
Undetectable	132.8±68.8	112.2±57.3	31.0±16.9	27.4±11.5	22.2±14.3	16.3±8.3	12.1±7.0

Values are mean± S.D. with 5 rats in each group. Sham : sham operated control rats; CBDL : common bile duct ligated rats.

Table 4. Activities of mitochondrial and microsomal monoamine oxidase A and B (MAO A and B) in cholestatic rat liver

Days following ligation	Mitochondrial MAO A		Mitochondrial MAO B (pmol ammonia $min^{-1}\cdot mg$ protein)		Microsomal MAO A		Microsomal MAO B	
	Sham	CBDL	Sham	CBDL	Sham	CBDL	Sham	CBDL
1(n=8)	1,278±270	1,104±251	1,630±310	1,298±283[a]	556±152	524±156	657±162	642±165
2(n=8)	1,290±265	1,120±243	1,628±312	1,186±252[a]	558±155	522±161	654±167	641±170
3(n=8)	1,282±258	814±211[b]	1,632±308	1,054±236[c]	563±146	562±158	661±159	637±167
7(n=8)	1,292±271	804±209[b]	1,620±305	980±224[c]	559±154	532±164	658±165	628±156
14(n=7)	1,294±263	544±147[c]	1,624±313	566±196[c]	561±157	392±127	649±155	528±142
28(n=7)	1,284±273	388±112[c]	1,626±316	322±141[c]	567±144	308±109[a]	645±152	406±131[a]
42(n=7)	1,289±258	370±107[c]	1,632±307	352±127[c]	565±142	292± 98[a]	648±158	410±118[a]

Values are mean± S.D. Sham : sham operated control rats; CBDL : common bile duct ligated rats.
Values significantly different from sham operated control values ([a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$).

Table 5. Monoamine oxidase A and B kinetic parameters

Cell fractions	K_m (mM)		V_{max} (pmol amoonia $min^{-1} \cdot mg$ protein^{-1})	
	Sham	CBDL	Sham	CBDL
MAO A				
Mitochondria	6.31± 1.32	6.76± 1.43	2,622± 575	1,079± 388^b
Microxome	9.14± 2.02	9.51± 1.89	1,220± 301	732± 186^a
MAO B				
Mitochondria	5.87± 0.94	6.22± 1.12	3,224± 708	1,062± 367^c
Microsome	8.45± 1.79	8.97± 1.56	1,371± 340	919± 223^a

Michaelis-Menten constants were determined using 5-hydroxytryptamine HCl as a substrate for MAO A and benzylamine HCl for MAO B from cholestatic rat livers at 28th day after CBD ligation. Values are mean± S.D. with 5 rats in each group. Sham: sham operated control rats; CBDL: common bile duct ligated rats. Values significantly different from sham operated control values ($^a p<0.05$, $^b p<0.01$, $^c p<0.001$).

Table 6. Activities of the serum monoamine oxidase (MAO) in cholestatic rat

MAO activities ($\Delta A \times 100$ $min^{-1} \cdot ml^{-1}$)							
Sham	CBDL 1st	2nd	3rd	7th	14th	28th	42nd day
Undetectable				7.3± 2.9	18.8± 4.5	15.6± 3.9	13.4± 5.4

Values are mean± S.D. with 5 rats in each group.
Sham: sham operated control rats; CBDL: common bile duct ligated rats.

Gaudin *et al.*, 1990; Gaudin *et al.*, 1991; Floras *et al.*, 1991).

To know the cause of the change of COMT and MAO activities, V_{max} and K_m values were studied. V_{max} values of COMT and MAO were decreased without the changes of K_m values. These results indicate that the synthesis of COMT and MAO is decreased in hepatobiliary disease.

Cytosolic COMT and MAO activities were decreased, while serum COMT and MAO activity were detected after CBD ligation without the coincidence in timing of changes. These results are suggestive that COMT and MAO in the liver flow into the blood through the damaged cell membranes caused by the CBD ligation. However, it is hard to explain from this experiment about the non-coincidence in the timing of the changes.

In summary, plasma catecholamine levels are increased in liver disease due to decreased hepatic degradation of catecholamines by decreased activities of COMT and MAO. Decreased activity of these enzymes are caused by decreased biosynthesis and by flowage into the blood from the damag ed hepatocyte.

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References

Baldessarini, R. J. (1991) In Goodman & Gilman's the Pharmacological Basis of Therapeutics, 8th ed. (Gilman, A. G., Rall, T. W., Nies, A. S., Taylor, P. eds.) pp. 415-418, Pergamon Press, New York.

Bertocci, B., Garotta, G., Prada, M. D., Lahm, H. W., Zürcher, G., Virgallita, G. and Miggiano, V. (1991) *Biochim. Biophys. Acta* **1080**, 103.

Borchardt, R. T. and Cheng, C. F. (1978) *Biochim. Biophys. Acta* **522**, 49.

Borchardt, R. T. (1981) *Method Enzymol.* **77**, 267.

Chang, D. S., Kwak, J. S. and Sohn, T. J. (1987) *The Kyungpook Univ. Med. J.* **28**, 113.

Floras, J. S., Legault, L., Morali, G. A., Hara, K. and Blendis, L. M. (1991) *Annu. Intern. Med.* **114**, 373.

Gaudin, C., Braillon, A., Selz, F., Cuche, J. L. and Lebrec, D. (1990) *J. Lab. Clin. Med.* **115**, 589.

Gaudin, C., Braillon, A., Poo, J. L., Moreau, R., Hadengue, A. and Lebrec, D. (1991) *J. Hepatol.* **13**, 161.

Ghosh, S. K. and Guha, S. R. (1977) *Indian J. Physiol. Pharmacol.* **21**, 147.

Granner, D. K. (1993) In Harper's Biochemistry, 23rd ed. (Murray, R. K., Granner, D. K., Mayer, P. A., Rodwell, V. W. eds.) pp. 536-541, Appleton & Lange, East Norwalk.

Greco, A. V., Ghirlanda, G., Bochicchio, G. B., Caputo, S., Uccioli, L. and Rebuzzi, A. G. (1985) *Minerva. Med.* **76**, 1911.

Hsu, C. T. (1992) *J. Auton. Nerv. Syst.* **37**, 163.

Kim, H. S., Park, J. Y., Kim, E. Y., Kwak, K. S., Choi, Y. H. and Chung, J. M. (1989) *Kor. J. Intern. Med.* **36**, 459.

Kountouras, J., Billing, B. H. and Scherer P. J. (1984) *Br. J. Exp. Pathol.* **65**, 305.

Kwak, C. S. and Kwak, J. S. (1986) *The Keimyung Univ. Med. J.* **5**, 45.

Nagatsu, T. and Yaki, K. (1966) *J. Biochem. (Japan)* **60**, 219.

McEwen, C. M. Jr. and Cohen, J. D. (1963) *J. Lab. Clin. Med.* **62**, 766.

Raxworthy, M. J. and Gulliver, P. A. (1986) *Biochim. Biophys. Acta* **870**, 417.

Tilgmann, C. and Kalkkinen, N. (1990) *FEBS Lett.* **264**, 95.