

# mRNA Expression of Enzymes Involved in Taurine Biosynthesis in Rat Adipose Tissues

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mRNA levels of the enzymes involved in taurine synthesis were compared among 12 rat tissues. Using a quantitative reverse transcription-polymerase chain reaction (RT-PCR) and Northern-blotting, high levels of mRNA for cysteine dioxygenase were confirmed in the liver and kidney. Unexpectedly, the mRNA levels in epididymal and perirenal white adipose tissues and interscapular brown adipose tissue were remarkably high, at least compared with those observed in the liver and kidney. Cysteine dioxygenase mRNA levels in other tissues were very low. Using Northern blotting, significant amounts of cysteine sulfinic acid decarboxylase mRNA were detected in the liver, kidney, epididymal and perirenal white adipose tissues, and brown adipose tissue, but not in other tissues. Again, the mRNA levels of cysteine sulfinic acid decarboxylase in adipose tissues were comparable to or even higher than those in the liver and kidney. The activity of cysteine sulfinic acid decarboxylase in white and brown adipose tissues was 50% to 80% of that in the liver and much higher than the values observed in kidney, lung, and brain. However, the occurrence of cysteine dioxygenase activity in adipose tissues was not confirmed.

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TAURINE IS ABUNDANT in various tissues in mammals and is considered to be involved in various physiologic functions.<sup>1</sup> However, the principal physiologic role of this compound and its biochemical basis are far from clear. Taurine is synthesized from sulfur amino acids. Cysteine dioxygenase (EC 1.13.11.20) and cyteinesulfinic acid decarboxylase (EC 4.1.1.29) are involved in the conversion of cysteine to taurine. A thiol group of cysteine is oxygenated to form cysteine sulfinic acid by a reaction catalyzed by cysteine dioxygenase. A reaction catalyzed by cysteine sulfinic acid decarboxylase, in turn, converts the cysteine sulfinic acid to hypotaurine. The hypotaurine formed is oxidized to taurine, presumably by a nonenzymatic process.<sup>2</sup> Activity and mRNA levels of these enzymes are high in liver and are easily modified by various physiologic and nutritional conditions in the rat.<sup>3-16</sup> Dietary sulfur amino acids profoundly affect these parameters,<sup>5-8,10-13</sup> and it is considered that these enzymes in liver play a crucial role in regulating the whole body sulfur amino acid level.<sup>2</sup> One of the most important physiologic functions of taurine in liver is the conjugation of bile acid. However, the nutritional conditions, which increase hepatic bile acid synthesis or the bile acid pool, rather decrease cysteine dioxygenase activity in the liver.<sup>6,9</sup> The presence of enzymes involved in taurine synthesis in many extrahepatic tissues has also been reported.<sup>13-26</sup> Taurine synthesis may serve to maintain the function of each tissue. Clarification of the tissue distribution of enzymes involved in taurine biosynthesis is essential to understand the physiologic roles of taurine in maintaining the function and development of organisms. However, studies to compare the distribution of enzymes involved in taurine synthesis among tissues are scarce. In this context, we compared the mRNA levels of cysteine dioxygenase and cysteine sulfinic acid decarboxylase among 12 tissues in rats and, unexpectedly, found that the mRNAs of these enzymes are highly expressed in white and brown adipose tissues.

## MATERIALS AND METHODS

### DNA Substrates for the Preparation of Probes to Detect mRNA

DNA fragments encoding cysteine dioxygenase, cysteine sulfinic acid decarboxylase, and glyceraldehyde-3-phosphate-dehydrogenase

(GAPDH) genes were generated by reverse transcription-polymerase chain reaction (RT-PCR) in a final volume of 0.1 mL using RNA isolated from the liver of a male Sprague-Dawley rat as a template.<sup>27,28</sup> DNA fragments were purified by agarose gel electrophoresis and used as the substrates to prepare cDNA probes labeled with alkaline-phosphatase or [ $\alpha$ -<sup>32</sup>P]deoxycytidine triphosphate (dCTP). The up- and downstream primers used were 5'-TCATCCGAATCTTGCATGAGC-3' and 5'-CAGGTCTTAGTTGTTCTCCAG-3' for cysteine dioxygenase (size of PCR product was 566 bp), 5'-GGATTGTCGTAGATGAGGCC-3' and 5'-TTATCTCCTCCACCAAGTACC-3' for cysteine sulfinic acid decarboxylase (1,110 bp), and 5'-TAGACAAGATGGTGAAGGTCG-3' and 5'-TCTCTTGTCTCAGTATCC-3' for GAPDH (1,058 bp), respectively, and 30 cycles of PCR were conducted to amplify these substrate DNAs. The primers were designed from rat cDNA sequences available in the GenBank database (accession numbers: cysteine dioxygenase, M35266; cysteine sulfinic acid decarboxylase, X94152; and GAPDH, X02232). The DNA sequences of the amplification products were partially determined by direct sequencing to confirm their identities with reported cDNA sequences of corresponding enzymes.

### Analysis of mRNA Levels of Cysteine Dioxygenase and Cysteine Sulfinic Acid Decarboxylase in Tissues

RNA was extracted from 12 tissues (brain, lung, spleen, heart, epididymal and perirenal white adipose tissues, interscapular brown adipose tissue, skeletal muscle, small intestine, kidney, liver, and testis) of 8-week-old male Sprague-Dawley rats (Charles River, Kanagawa, Japan) by the acid guanidium thiocyanate-phenol-chloroform method.<sup>27,28</sup> Quantitative RT-PCR to quantify cysteine dioxygenase and GAPDH mRNA levels in tissues was conducted in a final volume of 0.025 mL as detailed previously.<sup>29</sup> Primers used for PCR were the same as those used to generate

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DNA fragments to prepare labeled probes. After completion of PCR, 0.02 mL of the reaction mixture was heat-denatured and applied to a nylon membrane (Hybond-N<sup>+</sup>, Amersham International, Bucks, UK) using a dot-blot apparatus (Bio-Rad, Hercules, CA), and cross-linked by ultraviolet (UV) irradiation. Southern blot analysis was conducted by a standard procedure. The PCR mixtures (0.02 mL) were electrophoresed on a 1% agarose gel and transferred to a nylon membrane. The PCR products on the nylon membrane were hybridized with alkaline phosphatase-labeled cDNA probes and chemiluminescence was generated by CDP-Star detection reagent (Amersham International, Bucks, UK) and quantified by an imaging analyzer (Bio-Rad). For Northern blot analysis to detect mRNAs of cysteine dioxygenase and cysteine sulfinic acid decarboxylase, RNA samples from various tissues (30 g) were denatured and electrophoresed on a 1.1% agarose gel containing 0.66 mol/L formaldehyde. RNAs were transferred to a nylon membrane, fixed with UV irradiation, and hybridized with cDNA probe labeled with [ $\alpha$ -<sup>32</sup>P]dCTP specific to cysteine dioxygenase or cysteine sulfinic acid decarboxylase. After quantification of mRNA levels by an imaging analyzer, membranes were stripped of these enzyme probes and rehybridized with <sup>32</sup>P-labeled cDNA probe specific to 18S ribosomal RNA to control for the results obtained.

#### Analysis of Cysteine Sulfinic Acid Decarboxylase Activity in Tissues

Rat tissues (brain, lung, epididymal and perirenal white adipose tissues, interscapular brown adipose tissue, kidney, and liver) were homogenized in 0.25 mol/L sucrose containing 1 mmol/L EDTA and 3 mmol/L Tris-HCl (pH 7.2). A total of 7 and 5 mL sucrose solution was used for each gram of liver and kidney, respectively, and 3 mL for each gram was used for the other tissues. Homogenates were centrifuged at  $9,000 \times g$  for 10 minutes, and the supernatants were used as enzyme sources in assaying cysteine sulfinic acid decarboxylase activity as detailed previously.<sup>8</sup> The enzyme product, hypotaurine, was analyzed by high-performance liquid chromatography. Taurine concentrations in tissues were determined by high-performance liquid chromatography as described previously.<sup>9</sup>

#### Statistical Analysis

Data were analyzed by the method of Snedecor and Cochran,<sup>30</sup> and significant differences of the means were evaluated at the level of  $P < .05$ .

## RESULTS

RT-PCRs specific to mRNAs of cysteine dioxygenase and a housekeeping gene (GAPDH) in 12 tissues of rats were conducted using 5 to 50 ng RNA substrate and specific primers for 18 and 16 cycles, respectively (Figs 1 and 2). PCR products were dot-blotted on nylon membranes and analyzed. The values were expressed as a percentage, assigning the value obtained in liver using 50 ng RNA substrate as 100%. The results shown in these figures represent the means with their standard errors obtained from tissues of 3 rats. Under the respective RT-PCR conditions for cysteine dioxygenase and GAPDH, the intensity of the chemiluminescence increased linearly with respect to the amount of RNA substrate used in all tissues analyzed.

As expected, chemiluminescence signals obtained by RT-PCR specific to cysteine dioxygenase were strong in liver and kidney and weaker, but significant, in the brain and lung (Fig 1). Unexpectedly, signals detected in epididymal and perirenal white adipose tissues and interscapular brown adipose tissue were intense and at least comparable to those observed in the liver and kidney. Signals were almost negligible in spleen, heart, and small intestine, and some weak signals were detected in the muscle and testis.

In contrast to the situation for cysteine dioxygenase, RT-PCR detected GAPDH mRNA ubiquitously, although the values differed considerably among the tissues (Fig 2).

mRNA levels of cysteine dioxygenase and GAPDH in 12 tissues from 7 rats estimated by quantitative RT-PCR using 30

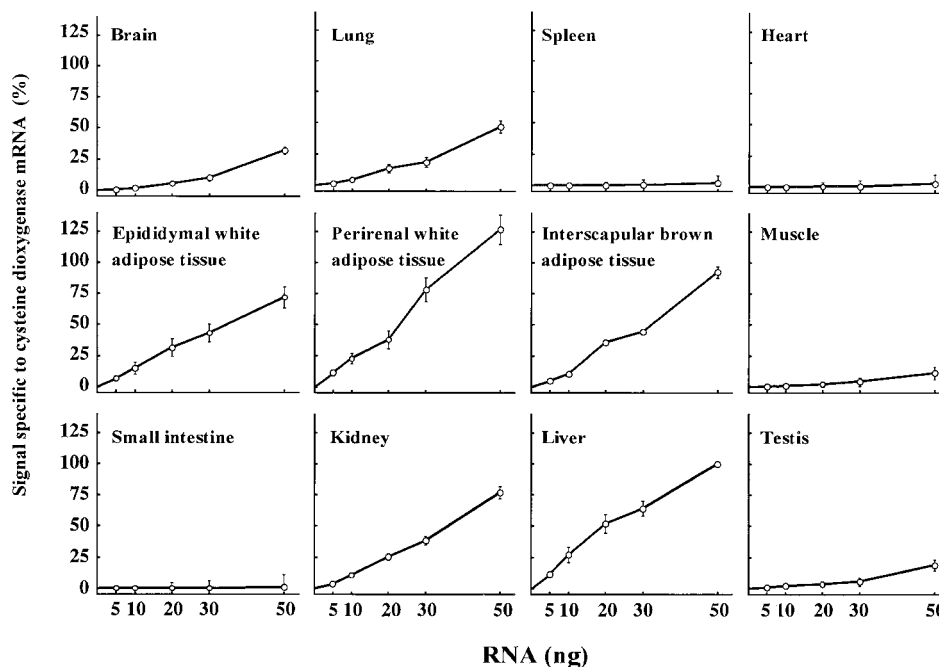
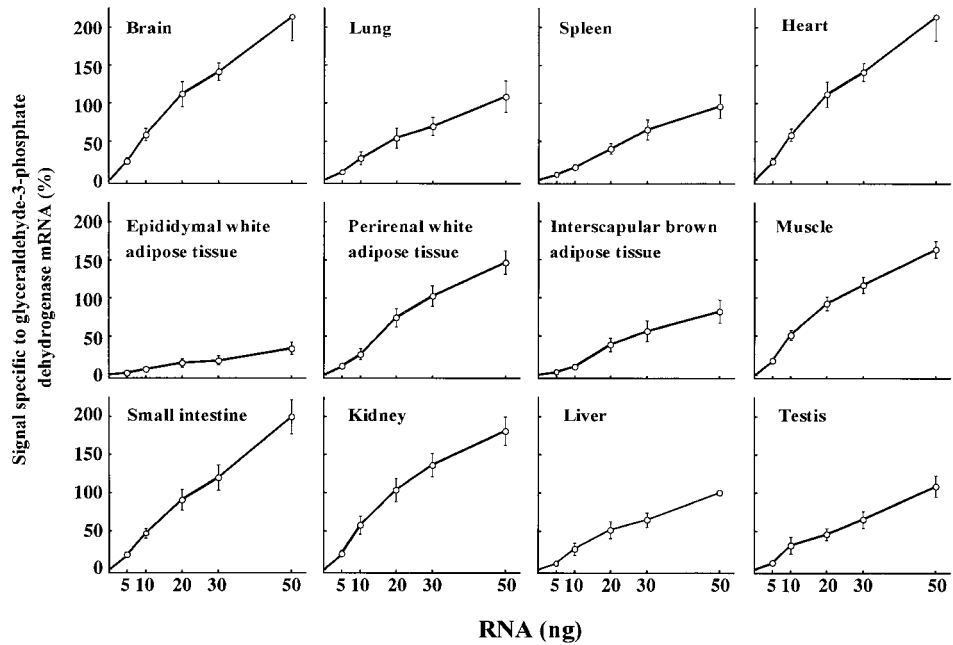


Fig 1. Chemiluminescence signal generated by RT-PCR specific to cysteine dioxygenase mRNA in rat tissues using varying amounts of RNA substrate. PCR products were dot-blotted on a nylon membrane and hybridized with alkaline phosphatase-labeled specific cDNA probe. Chemiluminescence was generated by CDP-Star detection reagent and quantified by an imaging analyzer. Values are expressed as a percentage assigning the value obtained in the liver using 50 ng RNA substrate as 100%. Each value represents the mean  $\pm$  SE for 3 rats.

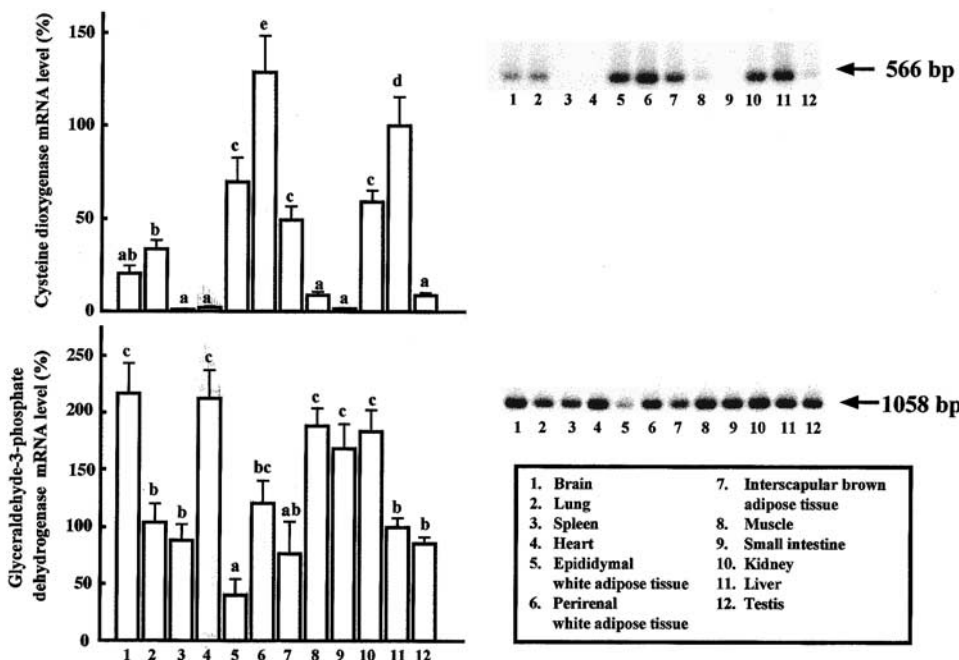


**Fig 2.** Chemiluminescence signal generated by RT-PCR specific to GAPDH mRNA in rat tissues using varying amounts of RNA substrate. PCR products were dot-blotted on a nylon membrane and analyzed as described in the legend to Fig 1. Values are expressed as a percentage assigning the value obtained in the liver using 50 ng RNA substrate as 100%. Each value represents the mean  $\pm$  SE for 3 rats.

ng RNA substrate are summarized in Fig 3. The values were expressed as a percentage, assigning the value in the liver as 100%. Data were analyzed statistically to show the differences in gene expression among the tissues. The data showed unequivocally that adipose tissues express cysteine dioxygenase mRNA at high levels. Among adipose tissues, the mRNA level was the highest in perirenal depot and significantly higher than the levels observed in the liver and kidney. Significant amounts of cysteine dioxygenase mRNA were also detected in the brain and lung, but considerably less than in the liver, kidney, and

adipose tissues. Apparently, the tissue distribution of RT-PCR signals specific to GAPDH mRNA was completely different from that of the signals specific to cysteine dioxygenase. The values among tissues were 40% to 200% of that in the liver. Figure 3 also shows representative results obtained by Southern blot hybridization. Analysis by this method detected a single band of expected size for RT-PCR of both cysteine dioxygenase (566 bp) and GAPDH (1,058 bp) and no extra band. This confirms the specificity of the method used.

To confirm the unexpected finding that cysteine dioxygenase



**Fig 3.** Cysteine dioxygenase and GAPDH mRNA levels examined by RT-PCR among rat tissues. RT-PCR was conducted using 30 ng RNA substrate. PCR products were dot-blotted on a nylon membrane and analyzed as described in the legend to Fig 1. Values are expressed as a percentage assigning the value obtained in the liver as 100%. Each value represents the mean  $\pm$  SE for 7 rats. Values not sharing a common letter are significantly different at  $P < .05$ . Typical results of Southern blot analysis of the products obtained by RT-PCR specific to cysteine dioxygenase and GAPDH mRNAs are also shown.

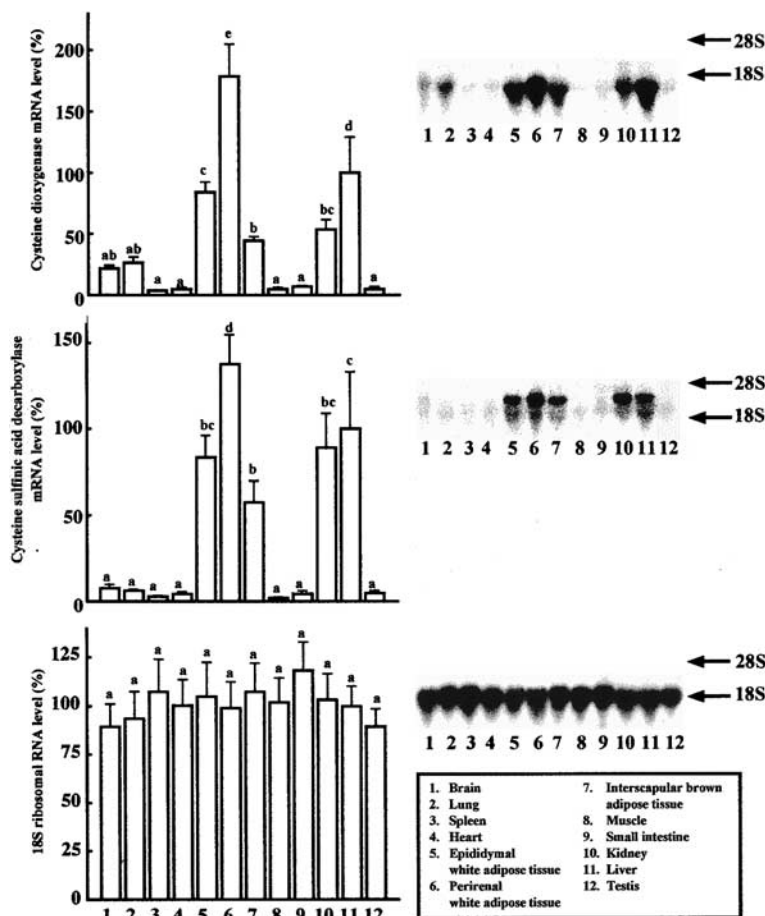


Fig 4. Northern blot analysis of cysteine dioxygenase, cysteine sulfinic acid decarboxylase mRNAs, and 18S ribosomal RNA among rat tissues. RNA samples (30 g) were denatured and electrophoresed on 1.1% agarose gel containing 0.66 mol/L formaldehyde, then transferred to a nylon membrane and fixed with UV irradiation. The RNA on nylon membranes was hybridized with radiolabeled cDNA probe specific to mRNA of cysteine dioxygenase or cysteine sulfinic acid decarboxylase. After quantification of mRNA levels, membranes were stripped of the enzyme probes and rehybridized with <sup>32</sup>P-labeled cDNA probe specific to 18S ribosomal RNA to control for the results obtained. Values are expressed as a percentage assigning the value obtained in the liver as 100%. Each value represents the mean ± SE for 7 rats. Values not sharing a common letter are significantly different at *P* < .05.

mRNA was highly expressed in adipose tissues, Northern blot analysis to quantify mRNA levels of cysteine dioxygenase and cysteine sulfinic acid decarboxylase was conducted (Fig 4). Membranes were rehybridized with a cDNA probe specific to 18S ribosomal RNA to control for the results obtained. The results obtained by Northern blotting of cysteine dioxygenase mRNA essentially confirmed those observed by quantitative RT-PCR. Significant signals for cysteine dioxygenase mRNA were detected in the brain, lung, adipose tissues, kidney, and liver, but not in the other tissues. The high expression level of cysteine dioxygenase mRNA in adipose tissues was confirmed. The mRNA levels in epididymal and perirenal white adipose tissues and interscapular brown adipose tissue were comparable to or even higher than those in the liver and kidney. Among the adipose tissues, the expression was greatest in perirenal white adipose tissue, being significantly higher than that in the liver.

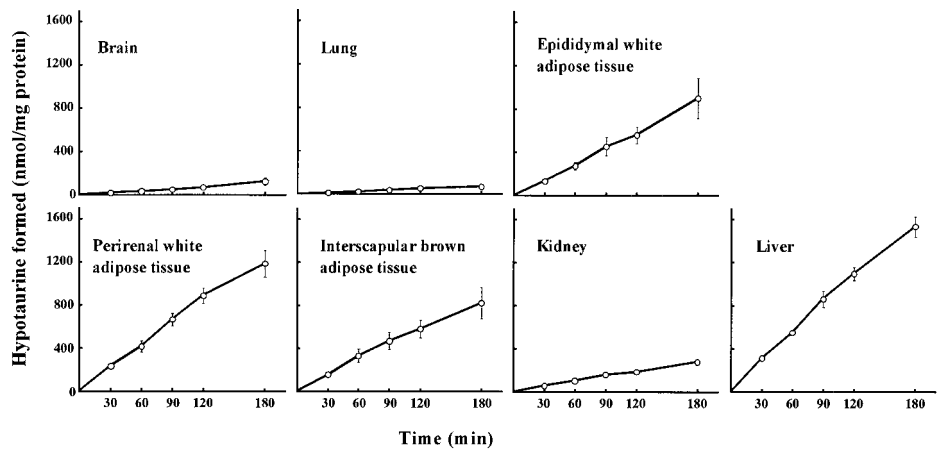
Significant amounts of cysteine sulfinic acid decarboxylase mRNA were detected in adipose tissues, kidney, and liver. Signals in the other tissues, including the brain and lung, were very weak. The level of expression of cysteine sulfinic acid decarboxylase mRNA in epididymal and perirenal white adipose tissues and interscapular brown adipose tissue was high. As in the case of the cysteine dioxygenase mRNA, the highest value among the adipose tissues was obtained in perirenal

depot. Levels of 18S ribosomal RNA were indistinguishable among tissues.

Cysteine sulfinic acid decarboxylase activity was examined in 7 tissues including brain, lung, epididymal and perirenal white adipose tissues, interscapular brown adipose tissue, kidney, and liver. The formation of the enzyme product, hypotaurine, was analyzed by high-performance liquid chromatography. The time-dependent production of hypotaurine in the enzyme reaction mixture from tissues of 3 individual rats is summarized in Fig 5. The production using enzyme preparations from various tissues increased linearly during 3 hours of incubation. The highest value was obtained in the liver. The enzyme activity in adipose tissues was somewhat lower than that in the liver, but much higher than that observed in the other tissues (brain, lung, and kidney). The enzyme activities using 2 hours of incubation and taurine concentrations in tissues from 7 rats are summarized in Table 1, and statistical examinations of the differences among tissues were made. It was confirmed that the activity was strongest in the liver. Although the activities were significantly lower in adipose tissues than in the liver, they were much higher than those observed in the brain, lung, and kidney. Taurine concentrations were lower in white and brown adipose tissues than in the other tissues. Apparently, no correlation exists between cysteine sulfinic acid decarboxylase activity and taurine concentrations in tissues.



**Fig 5. Hypotaurine formation in rat tissue homogenates as a function of incubation time.** Each value represents the mean  $\pm$  SE for 3 rats. The 9,000  $\times$  g supernatant fraction of tissue homogenates was used as an enzyme source. The amounts of protein used were (in milligrams): brain, 0.40 to 0.49; lung, 0.94 to 1.05; epididymal white adipose tissue, 0.15 to 0.23; perirenal white adipose tissue, 0.11 to 0.18; interscapular brown adipose tissue, 0.16 to 0.30; kidney, 0.39 to 0.44; and liver, 0.14 to 0.18, respectively.



In a preliminary experiment, we analyzed cysteine dioxygenase activity in the perirenal and epididymal white adipose tissues, kidney, and liver. A 9,000  $\times$  g supernatant fraction of tissue homogenates (3.0 to 3.4 mg protein for liver and kidney and 0.6 to 0.7 mg for adipose tissues) was incubated at 37°C for 2 hours in the presence of cysteine substrate, and the enzyme products were analyzed by high-performance liquid chromatography as described previously.<sup>6,9</sup> Cysteine sulfinic acid, the enzyme product of cysteine dioxygenase, its oxidation product (cysteic acid) and hypotaurine were detected in the reaction mixture using the enzyme sources from liver and kidney. However, no detectable amounts of these metabolites were found using the enzyme sources from white adipose tissues (data not shown).

**DISCUSSION**

There is a general consensus that the liver is the major site of taurine synthesis in the rat. Previous studies also indicated that taurine synthesis occurs in tissues other than the liver. However, studies to compare the abundance of activity and/or mRNA of the enzymes involved in taurine biosynthesis among tissues are scarce. The information regarding the tissue distribution of cysteine dioxygenase is rather controversial. High-enzyme activity has been detected in the liver, and it is well demonstrated that various physiologic and nutritional conditions affect the activity.<sup>3-6,9,11-16</sup> Previous studies also detected enzyme activity in many extrahepatic tissues including kid-

ney,<sup>13,15,16</sup> brain,<sup>13-16,24,26</sup> retina,<sup>26</sup> reproductive tissues,<sup>24</sup> heart,<sup>14-16</sup> stomach,<sup>15</sup> and spleen.<sup>15</sup> Several earlier studies<sup>13-16</sup> indicated that the activity in many extrahepatic tissues is as high as that observed in the liver. However, recent studies<sup>19,20</sup> indicated that the tissue distribution of cysteine dioxygenase mRNA is restricted in the rat. Tsuboyama et al<sup>19</sup> examined cysteine dioxygenase mRNA abundance among brain, heart, kidney, liver, lung, skeletal muscle, testis, and thymus in the rat. They showed that the mRNA was detectable in the brain, kidney, liver, and lung, but not in the other tissues. The mRNA level was highest in the liver, with the value in both kidney and lung 40% and in brain 12% of that in the liver. The present study comparing the mRNA levels of cysteine dioxygenase mRNA by RT-PCR and Northern blot hybridization also indicated that the tissue distribution of enzyme mRNA is rather restricted in the rat. Our results essentially confirmed the observation made by Tsuboyama et al<sup>19</sup> that cysteine dioxygenase mRNA is highly expressed in the liver and kidney and moderately expressed in the lung and brain. In addition, we found that cysteine dioxygenase mRNA is highly expressed in adipose tissues, and the mRNA level in these tissues is as high as that in the liver.

With regard to the tissue distribution of cysteine sulfinic acid decarboxylase activity, Yamaguchi et al<sup>15</sup> compared the enzyme activity among 7 rat tissues including brain, heart, stomach, kidney, spleen, intestine, and liver. They showed that cysteine sulfinic acid decarboxylase activity was strongest in the liver and moderate, but significant, in the brain and kidney. The levels of activity were much lower in the other tissues. The presence of the activity and/or mRNA in liver, kidney, and brain has been confirmed by many other investigators,<sup>7-11,13-18,21-23</sup> and it has been considered that brain and kidney, in addition to the liver, are sites of taurine biosynthesis in the rat.<sup>2</sup> In a recent study using RT-PCR, Hu et al<sup>25</sup> proved the existence of cysteine sulfinic acid decarboxylase mRNA in the mammary gland. The intensity of the signal obtained from this tissue was comparable to that from the liver. However, the linearity of RT-PCR to quantify cysteine sulfinic acid decarboxylase mRNA has not been well certified under that set of experimental conditions. Therefore, the significance of this finding remains to be clarified. In the present study using

**Table 1. Cysteinesulfinic Acid Decarboxylase Activity and Taurine Concentration in Rat Tissues**

	Cysteinesulfinic Acid Decarboxylase (nmol/min/mg protein)	Taurine ( $\mu$ mol/g)
Brain	0.46 $\pm$ 0.07 <sup>a</sup>	5.06 $\pm$ 0.23 <sup>d</sup>
Lung	0.29 $\pm$ 0.04 <sup>a</sup>	4.22 $\pm$ 0.11 <sup>c</sup>
Epididymal white adipose tissue	4.19 $\pm$ 0.34 <sup>b</sup>	0.641 $\pm$ 0.044 <sup>a</sup>
Perirenal white adipose tissue	5.88 $\pm$ 0.61 <sup>c</sup>	0.693 $\pm$ 0.079 <sup>a</sup>
Interscapular brown adipose tissue	3.69 $\pm$ 0.55 <sup>b</sup>	1.96 $\pm$ 0.21 <sup>b</sup>
Kidney	1.29 $\pm$ 0.15 <sup>a</sup>	4.26 $\pm$ 0.24 <sup>c</sup>
Liver	7.26 $\pm$ 0.89 <sup>d</sup>	6.52 $\pm$ 0.59 <sup>a</sup>

NOTE. Values in a column not sharing a common superscript letter are significantly different at  $P < .05$ .

Northern blot hybridization, we confirmed that cysteine sulfinic acid decarboxylase mRNA is highly expressed in the liver and kidney. As in the case of cysteine dioxygenase mRNA, we found that mRNA levels of cysteine sulfinic acid decarboxylase in epididymal and perirenal white adipose tissues and in brown adipose tissue were comparable to or even higher than those observed in the liver and kidney. We could not find significant signals for the mRNA in the brain and lung. The levels in these tissues may be lower than the detection limit of Northern blot hybridization. Tappaz et al<sup>22</sup> reported that cysteine sulfinic acid decarboxylase mRNA was detectable in the liver, kidney, and brain, but not in the heart by Northern blot hybridization. The analysis of 2.5  $\mu\text{g}$  poly(A<sup>+</sup>)-mRNA or 5 to 20  $\mu\text{g}$  total RNA was sufficient to detect the enzyme mRNA in the liver and kidney. Detection of the mRNA in the brain, however, required as much as 10  $\mu\text{g}$  poly(A<sup>+</sup>)-mRNA, and no signals were detected using 5 to 20  $\mu\text{g}$  total RNA. It is, therefore, apparent that the level of cysteine sulfinic acid decarboxylase mRNA in the brain is too low to be detected by the conventional method. Consistent with this notion, the enzyme activity in this tissue was less than one tenth of that observed in the liver in the present study. The analysis of cysteine sulfinic acid decarboxylase activity among tissues also provided evidence that this enzyme is highly expressed in adipose tissues. Although the enzyme activity in adipose tissues was significantly lower than that in the liver, it was much higher than the activity in the other extrahepatic tissues. It is, therefore, apparent that this enzyme is highly expressed, not only as mRNA, but also as protein in adipose tissues. Despite that the cysteine dioxygenase mRNA levels in adipose tissues were as high as those observed in the liver, attempts to detect cysteine dioxygenase activity in adipose tissues have, so far, been unsuccessful. Therefore, there is a possibility that the cysteine dioxygenase mRNA expressed in adipose tissues is not sufficiently translated to form the enzyme protein. In this context, Bella et al<sup>11</sup> provided evidence that the expression of cysteine dioxygenase in the liver is regulated primarily at the level of translation rather than transcription. In the case of hepatic cysteine sulfinic acid decarboxylase, it has been reported that the enzyme activity and protein content well paralleled the mRNA level.<sup>10</sup> Alternatively, there is the possibility that the cysteine dioxygenase in adipose tissues is inactivated during the preparation of the enzyme source. Therefore, efforts to prove the existence of cysteine dioxygenase protein and/or activity in adipose tissues are still required to clarify the role of adipose tissues in taurine synthesis.

The observations of the current study that mRNAs for enzymes involved in taurine biosynthesis are highly expressed in

adipose tissues possibly indicate that adipose tissues are the sites where taurine is actively synthesized in the rat. We also demonstrated that the level of cysteine sulfinic acid decarboxylase activity in these tissues is as high as that in the liver. However, the failure to detect cysteine dioxygenase activity in adipose tissues is not consistent with this consideration. In addition, the high expression level of enzymes involved in taurine synthesis is associated with a high rate of taurine synthesis only under conditions where sufficient amounts of sulfur amino acid substrates are available in the tissue. Therefore, a study to estimate the actual *in vivo* rate of tissue taurine synthesis is required to draw a definite conclusion regarding the role of adipose tissues in taurine synthesis.

The present observation that taurine concentrations were considerably lower in white and brown adipose tissues than in the other tissues may go against the consideration that adipose tissues are the sites of active taurine synthesis. However, endogenous taurine biosynthesis is not the sole determinant in regulating intracellular taurine accumulation. Another crucial factor modifying tissue taurine concentrations is the uptake of exogenous taurine through a specific transporter.<sup>1</sup> In fact, studies have indicated that an alteration in the expression of the taurine transporter modifies intracellular taurine concentrations.<sup>31-33</sup> There is a possibility that the expression of the taurine transporter is weak and thus gives low tissue taurine levels in adipose tissues despite that active taurine synthesis occurs in these tissues. Examinations in rats,<sup>34</sup> mice,<sup>35</sup> dogs,<sup>36</sup> and humans<sup>37</sup> showed that taurine transporter mRNA levels differ considerably among tissues. However, information regarding the taurine transporter protein or mRNA level in adipose tissues has been lacking. Therefore, clarification of the tissue distribution of the taurine transporter, in addition to that of enzymes involved in taurine synthesis, is required to understand the mechanism regulating tissue taurine levels.

In conclusion, the present study provided evidence that mRNAs of cysteine dioxygenase and cysteine sulfinic acid decarboxylase, the enzymes involved in taurine biosynthesis, are highly expressed in white and brown adipose tissues of rats. Values ranging from 50% to 180% of that in liver were obtained depending on the analysis method used or location of the depot examined. We also demonstrated the occurrence of cysteine sulfinic acid decarboxylase activity in white and brown adipose tissues. The level of enzyme activity was 50% to 80% of that in the liver and much higher than the levels observed in other extrahepatic tissues. However, we could not confirm the occurrence of cysteine dioxygenase activity in adipose tissues. Therefore, further study is required to clarify the role of adipose tissues in taurine biosynthesis.

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