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Taurine Synthesis, Concentration, and Bile Salt Conjugation in Rat, Guinea Pig, and Rabbit (38455)

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One of the more important metabolic pathways for the synthesis of taurine in mammals involves the decarboxylation of cysteinesulfinic acid (CSA) to hypotaurine or of cysteic acid (CA) to taurine by L-cysteinesulfinic carboxylyase [E.C.4.1.1.29] (1, 2). The level of this enzyme and the concentration of taurine in tissues of different species vary (1–4). Although direct comparisons have not been made, the level of decarboxylase activity[1] in the liver of different species appears to be related to the percentage of conjugated bile salts that each species conjugates with taurine (%T). However, the concentration of taurine in the livers of these species does not seem to correlate with the reported liver decarboxylase activity nor with the bile salt conjugation pattern (1). The purpose of the present study was to directly compare hepatic taurine synthesis capacity via the CSA–CA decarboxylase systems, taurine concentration, and the bile salt conjugation pattern in the rat, guinea pig, and rabbit. The relationship between the decarboxylase activity and the taurine concentration in the kidney, brain, lung, and heart of these species was also studied.

Material and Methods. Tissues from young adult male rats, guinea pigs and rabbits[2] were assayed for taurine and CSA and CA decarboxylase activities. Prior to sacrifice, the animals were maintained for at least 10 days in air-conditioned rooms and allowed to consume their respective commercial diets[3] and water *ad libitum*. Nonfasted animals were guillotined as needed to provide fresh tissues. At least two tissues from each animal were assayed in duplicate at each sacrifice. The liver, heart, kidneys, lungs, or brain were removed, weighed and a 20% w/v homogenate made using a Potter–Elvehjem apparatus and 0.067 *M* phosphate buffer (pH 6.8) containing dithiothreitol (1 m*M*). Separate aliquots of each homogenate were used for each of the three analyses.

CSA and CA decarboxylase activities were measured manometrically in a standard Warburg apparatus (5) under optimum conditions. The main compartment of each flask contained 600 mg tissue (3 ml homogenate), 0.5 nmole pyridoxal-5′-phosphate (0.05 ml of 10 μM solution), and 3 μmoles dithiothreitol (supplied in the phosphate buffer used for the homogenate). The side arm of each flask contained 0.2 ml of 0.16 *M* cysteinesulfinic acid or cysteic acid. When mixed with the contents of the main compartment, final substrate concentration was 0.01 *M*. The flasks were incubated at 37° under nitrogen. After an equilibration period of 15 min, the reaction was started by tipping the substrate out of the side arm. CO_2 evolution was measured at 5-min intervals during a 30-min incubation period when liver samples were assayed, and at 15-min intervals during a 90-min incubation period for all other tissues. The rate of CO_2 evolution was determined graphically and the amount of decarboxylase activity was obtained from the linear portion of each curve. This equates to the initial velocity of the reaction and,

[1] The term decarboxylase activity as defined here is the sum of E.C.4.1.1.29 activity with CSA as substrate plus E.C.4.1.1.29 activity with CA as substrate.

[2] Male, 130–150 g Wistar derived rats were obtained from Harlan Industries, Cumberland, IN; male, 450–550 g Hartley guinea pigs were obtained from Edmondson Small Animals, Washington, IN; male, 1.8–2.2 kg New Zealand rabbits were obtained from Wilson Small Animal Farm, Vincennes, IN.

[3] Purina Rat Chow, Purina Guinea Pig Chow and Purina Rabbit Chow obtained from Ralston–Purina, St. Louis, MO.

according to Michaelis–Menten kinetics, is proportional to the concentration of enzyme(s) (6).

Taurine in the tissues was measured as previously reported (7), using a modified method of Ling *et al.* (8).

Gall bladder bile was collected from rabbits and guinea pigs at the time of sacrifice; rat bile was obtained by ligating the duodenum as described by Hagerman and Schneider (9). The amount of bile salts conjugated with taurine (T) or glycine (G) was determined by tlc and reflectance densitometry (9) and the percent conjugated with taurine was calculated by: $\%T = (T \div (T+G)) \times 100$.

Results. Taurine conjugates accounted for 87 ± 6%[4] of the conjugated bile salts in rat bile. The ratio of glycine conjugates to taurine conjugates (G/T) was 0.15. The corresponding values for guinea pig bile and rabbit bile were 21 ± 14% (G/T of 3.8) and 6 ± 1% (G/T of 17), respectively. Haselwood (10) reported similar bile salt conjugation ratios for these three species.

The percent of bile salt conjugated with taurine was significantly correlated with the amount of taurine that could be synthesized per gram of liver (Fig. 1). However, the %T did not significantly correlate with the hepatic concentration of taurine (Table I) nor with the total hepatic synthesizing capacity for taurine via these (CSA–CA decarboxylase) pathways.

The relative levels of taurine synthetic capacity for the tissues examined were generally kidney > brain ≥ lung > heart, with the ranking of liver being variable (Table I). In the rat, the liver contained more decarboxylase activity than the other tissues. In the guinea pig, the kidney and liver contained the highest and equivalent levels of decarboxylase activity, but due to its greater mass, the liver had a larger synthetic capacity than the kidney. In the rabbit, the kidney, brain, and lung contained higher levels of decarboxylase activity than the liver. Although its concentration of decarboxylase activity was low, the rabbit liver had a taurine synthetic capacity equivalent to kidney. Essentially no decarboxylase activity was found in the heart in any of these species.

As with biliary %T, no correlation was observed between the level of taurine synthetic capacity (via CSA–CA decarboxylase pathway) and the concentration of taurine in the tissues (Table I). However, tissues with highest concentrations of taurine tended to be those with the lowest decarboxylase concentrations and the tissues with lower taurine concentrations tended to have higher decarboxylase concentrations. In the rat, guinea pig, and rabbit, the concentration of taurine in the heart was several fold greater than in any other tissue. The rat lung also contained a high concentration of taurine (Table I).

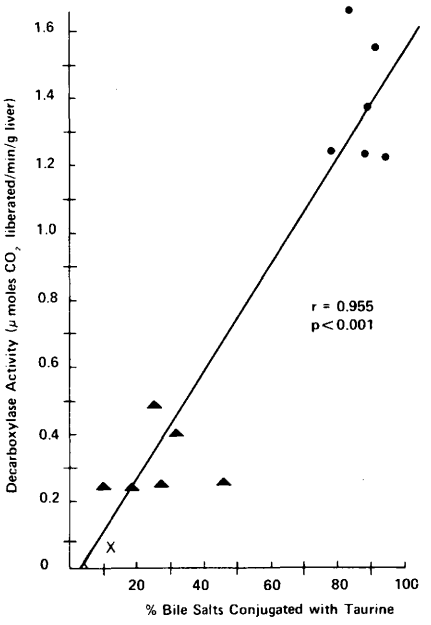

FIG. 1. Intraspecies correlation between hepatic CSA–CA decarboxylase activity and the percentage of bile salts in bile that are conjugated with taurine. The individual values are shown for (●) rat, (▲) guinea pig, and (×) rabbit.

Discussion. The correlation demonstrated between %T and CSA–CA decarboxylase activity among these species suggests that the interspecies parameters that affect %T also affect CSA–CA decarboxylase. Thus, each rat had higher %T and higher CSA–CA decarboxylase activity relative to any guinea pig, which had higher values in both parameters relative to any rabbit, which had low %T and low CSA–CA decarboxylase.

In comparing the taurine synthetic capacity of the tissues in the three species, the relative capacities of the liver and kidney are the most striking. The rat, whose bile contained a high %T, had a large hepatic capacity for taurine synthesis, but a low renal capacity. The guinea pig and rabbit, whose biles contained a much

[4] Mean ± SD, $n = 6$.

TABLE I. Comparison of Taurine Synthetic Capacity and Taurine Concentration in Rat, Guinea Pig, and Rabbit Tissues.

Tissue	Decarboxylase activity*		Taurine synthetic capacity**		Taurine concentration
	CSA	CA			
	μl CO_2/min/g		μmole/min/g	μmole/min/organ	μmole/g
Rat					
Liver	32.1 ± 4.3***,a	3.7 ± 0.7[a]	1.41	16.92	4.0 ± 1.6[a]
Kidney	3.5 ± 0.4[b]	0.6 ± 0.6[b]	0.16	0.31	7.1 ± 0.7[b]
Brain	2.1 ± 0.2[c]	0.6 ± 0.1[b]	0.11	0.20	5.0 ± 0.8[a]
Lung	0.4 ± 0.1[d]	0.1 ± 0.1[c]	0.02	0.02	10.3 ± 0.9[c]
Heart	0.0 ± 0.1[e]	0.0 ± 0.1[c]	0.00	0.00	20.4 ± 3.3[d]
Guinea Pig					
Liver	7.1 ± 2.4[a]	0.9 ± 0.3[a]	0.31	4.71	1.2 ± 0.3[a]
Kidney	8.6 ± 0.8[a]	1.0 ± 0.2[a]	0.38	1.13	2.7 ± 0.6[b]
Brain	1.9 ± 0.9[b]	0.8 ± 0.9[a,b]	0.11	0.24	1.3 ± 0.5[a]
Lung	2.6 ± 0.7[b]	0.5 ± 0.4[b]	0.12	0.32	3.8 ± 0.8[c]
Heart	0.3 ± 0.1[c]	0.0 ± 0.1[c]	0.01	0.01	11.1 ± 1.0[d]
Rabbit					
Liver	0.9 ± 0.3[a]	0.1 ± 0.1[a]	0.04	3.52	3.0 ± 1.5[a]
Kidney	5.7 ± 0.6[b]	0.3 ± 0.0[b]	0.24	3.91	3.6 ± 1.0[a]
Brain	1.4 ± 0.3[c]	0.3 ± 0.0[b]	0.07	0.52	1.6 ± 0.0[b]
Lung	1.2 ± 0.3[a,c]	0.1 ± 0.1[a]	0.05	0.61	2.9 ± 0.9[a]
Heart	0.1 ± 0.1[d]	0.0 ± 0.2[a]	0.00	0.00	10.8 ± 4.9[c]

* Results presented are from the data obtained with cysteinesulfinic acid (CSA) or cysteic acid (CA) as substrates.

** Based on taurine synthesis only from CSA plus CA, and calculated using $n = PV/RT$, where V equals sum of μl CO_2 produced per min from the two substrates, $P = 1$ atm, $T = 308°K$, and $R = 0.082$ μl atm $\mu mole^{-1} deg^{-1}$. The μmole/min/g are multiplied by the wet wt of tissue to yield μmole/min/organ. Final values are not statistically analyzed.

*** Mean ± SD, $n = 6$. Values in same group with same superscript (a–e) are not significantly different ($P > 0.05$) according to Scheffe's test (20).

lower %T, had moderate capacity for taurine synthesis in both liver and kidney. The renal synthetic capacity of both the guinea pig and rabbit was greater than that of the rat, while their hepatic capacities were much lower. The increased taurine synthetic capacity in extrahepatic tissues could be due to the lowered taurine supply from the liver and an effort to maintain concentrations of taurine in tissues which do not appear to synthesize taurine by this pathway, such as the heart.

Previously, Agrawal *et al.* (14) reported a lack of correlation between taurine concentration and CSA–CA decarboxylase activity in whole rat brain or its subcellular fractions. Similarly, data obtained in this and prior studies (8) suggested that there is no significant correlation between the concentration of taurine, the level of CSA–CA decarboxylase activity, and the turnover rate of taurine in any tissue from the rat. That the rates of taurine turnover, concentration, and synthesis in rat tissues seem independent of one another supports the hypothesis that taurine synthesized in one tissue may be conserved and utilized by other tissues.

The calculated amount of taurine that each species could synthesize (Table I) greatly exceeds the reported daily urinary losses for that species (1). This suggested that each species has the capability of synthesizing sufficient taurine via the CSA–CA decarboxylase pathway to maintain taurine balance when fed a chow diet. These estimated synthetic capacities are based on short-term optimum *in vitro* conditions, which probably do not exist physiologically for prolonged periods, and do not include all possible pathways (1, 19).

Whether E.C.4.1.1.29 is a single enzyme or a group of similar enzymes has not yet been definitely determined (2, 15–18). Therefore, the decarboxylase activity of a tissue in this report was based on the sum of the decarboxylation rates of

CSA and CA. Each tissue from each species had a different ratio of the rates of decarboxylation of the two substrates. This supports the suggestion that E.C.4.1.1.29 may be a family of enzymes (cf. 2, 15–18). Since all the tissues tested decarboxylated CSA at a greater rate than CA, CSA seems to be the preferred substrate, as suggested by Bergeret *et al.* (15).

Summary. The percentage of bile salts conjugated with taurine in bile (%T) , the taurine concentration, and the CSA–CA decarboxylase activity in the liver, kidney, brain, lung, and heart were compared in rats, guinea pigs, and rabbits. Hepatic CSA–CA decarboxylase and %T exhibited a highly significant ($P < 0.001$) positive correlation between the species tested. Species with lower hepatic taurine synthetic capability (and lower %T) had a greater extrahepatic taurine synthetic capability. No significant correlation was observed between taurine synthetic capacity (via CSA–CA decarboxylase), taurine concentration, and taurine turnover in the tissues of these species.

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