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INHIBITION OF THE SYNTHESIS OF TAUROCHOLIC ACID BY STRUCTURAL ANALOGUES OF TAURINE

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Abstract—Structural analogues of taurine that are inhibitors of the rat liver microsomal synthesis of taurocholic acid are reported. Isethionic acid, the hydroxy analogue of taurine, is the most potent inhibitor (I_{50} = 110 μM). Aminomethanesulfonic acid, a taurine analogue which has a single carbon bridge between the amino and sulfonic acid moieties, is also a very potent compound (I_{50} = 688 μM). Hydroxypropanesulfonic acid (I_{50} = 3125 μM), the higher homologue of isethionic acid, and aminoethane-sulfuric acid (I_{50} = 3750 μM) are considerably less potent inhibitors. Hypotaurine (I_{50} = 7500 μM) and glycine (I_{50} = 8800 μM), both substrates for the enzyme system, decrease taurocholate formation but only when used at extremely high concentrations.

The activation of cholic acid and subsequent conjugation of taurine with cholyl CoA by liver microsomes to form taurocholic acid were independently demonstrated in 1955 and 1956 by Bremer [1], Elliott [2] and Siperstein and Murray [3].

$$\text{Cholic acid} + \text{coenzyme A} + \text{ATP} \xrightarrow{Mg^{2+}} \text{cholyl CoA} + \text{AMP} + PP_i$$

$$\text{Cholyl CoA} + \text{taurine} \longrightarrow \text{taurocholic acid} + \text{coenzyme A}$$

Moreover, it has also been determined that other amino acids, e.g. glycine and hypotaurine, also form a peptide bond with cholic acid [4, 5]. Furthermore, experiments performed in our laboratory [6] indicate that the hydroxyl analogue of taurine, i.e. isethionic acid, forms an ester derivative with cholic acid. In this paper, experiments are presented which describe the inhibition of taurocholate synthesis by structural analogues of taurine.

MATERIALS AND METHODS

Materials. All solutions were prepared from reagent grade chemicals in either deionized or glass-distilled water. Taurine, cysteic acid, β-alanine, cysteine, cysteinesulfinic acid, aspartic acid, and glutamic acid were supplied by Sigma Chemical Co. Isethionic acid (K^+ salt) was supplied by Eastman Kodak Co. Aminomethanesulfonic acid, hydroxypropanesulfonic acid, and 2-aminoethylhydrogensulfate were purchased from Aldrich Chemical Co. Hypotaurine was obtained from CalBiochem. Ethanesulfonic acid was purchased from Pfaltz & Bauer, Inc. Coenzyme A, cholic acid, adenosine 5′-triphosphate, and dithiothreitol were purchased from P-L Biochemicals. [^{35}S]taurine (108 mCi/m-mole) was supplied by Amersham/Searle Corp. Sprague–Dawley male rats (125–200 g) were purchased from Sprague–Dawley, Madison, WI.

Preparation of rat liver microsomes. The microsomes were prepared according to the procedure of Elliott [7] with certain modifications. Male Sprague–Dawley rats were killed by cervical fracture. The liver was immediately removed, washed in cold distilled water, and them homogenized in 4 vol. of cold 0.25 M sucrose in a motor driven Teflon–glass homogenizer. The homogenate was then centrifuged for 20 min at 12,000 *g* (precipitate discarded) and for 30 min at 105,000 *g*. The microsomal pellet was suspended in cold, glass-distilled water (1 ml for each 1 g wet weight of liver). The microsomal suspension was homogenized in a Teflon–glass homogenizer with the dropwise addition of Triton X-100 (50 μl for every 1 ml of microsomal preparation), which resulted in a relatively clear solution. The enzyme preparation, stored in small aliquots at −15°, was stable for about 1 month without significant loss of activity. Protein was determined by the method of Lowry *et al.* [8].

Assay for the conjugation of taurine. The conjugation of taurine with cholic acid to form taurocholic acid was assayed according to the procedures of Elliott [7] and Bremer [9] with certain modifications. The reaction vessel (12-ml heavy-walled conical test tube) contained the following components (in μmoles) in a final volume of 0.16 ml: potassium phosphate buffer, pH 7.4, 20.0; magnesium sulfate, 0.6; ATP, 1.0; cholic acid, 0.1; coenzyme A, 0.055 (corrected for 92 per cent purity); dithiothreitol, 0.15; [^{35}S]taurine (containing 648,000 cpm), 0.003 to 0.008; and rat liver microsomes (110 μg). The reaction was terminated by dilution with 2.5 ml of 0.1 M H_2SO_4. *n*-Butanol (1 ml) was added; the aqueous–*n*–butanol mixture was thoroughly mixed on a Vortex Jr. for 30 sec to extract the radioactive taurocholic acid into the organic phase, and then an aliquot of the *n*-butanol layer was pipetted into 5 ml of Bray's [10] scintillation fluid.

Conditions of linearity as a function of time and microsomal protein were established for the enzymatic reaction before any experiments involving taurine analogues were performed.

Determination of inhibitor potency. The inhibitor potency of the taurine analogues was determined by comparison of the quantity (reaction rate) of taurocholic acid formation (calculated as a percentage) in the presence and in the absence of the anlogue. The

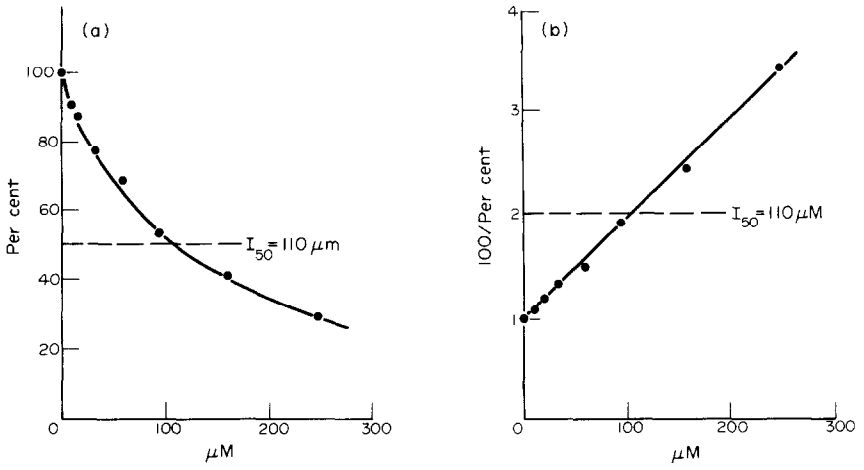

Fig. 1. Inhibition of taurocholic acid formation by isethionic acid. Taurine concentration was kept constant at 130 μM. The incubation was for 30 min at 37°. The components of the reaction system are described in Materials and Methods. (A) The reaction rate is expressed as 100 per cent in the absence of isethionic acid. Various concentrations of isethionic acid were added to the incubation system and the activity is expressed as a per cent of the uninhibited reaction. (B) The reciprocal of the reaction rate (100/per cent) is graphed as a function of the isethionic acid concentration.

taurine concentration was kept constant at 130 μM, which was the minimum concentration that was obtainable due to the quantity of endogenous taurine contained in the microsomal enzyme preparations (12.8 to 17.8 nmoles) and in the radioactive taurine (3–8 nmoles). A minimum concentration of taurine (130 μM) was utilized in the incubation system, since low levels of substrate magnified inhibitor potency. Taurine content was determined by either the amino acid analyzer procedure or by a double isotope enzymatic procedure [11].

The amount of inhibition was determined graphically by plotting the activity (per cent) or its reciprocal (100/per cent activity) [12, 13] as a function of the inhibitor concentration. Thus, for the taurine analogues that were inhibitors, the I_{50} value (concentration of inhibitor giving 50 per cent of control) is reported.

Determination of apparent kinetic inhibition constants. The dependence of the reaction rate on the concentration of taurine was examined in the presence and absence of isethionic acid and aminomethanesulfonic acid. Apparent kinetic constants were estimated by the Lineweaver and Burk [14] graphical representation of the data.

RESULTS AND DISCUSSION

The compounds which have been determined to decrease the incorporation of [^{35}S]taurine into taurocholic acid are close structural analogues of taurine. The possible mechanisms by which these compounds may accomplish the decrease in taurocholate formation are the following: (a) inhibition of the enzymatic reaction because of structural similarity to taurine; (b) formation of cholyl derivatives if the compounds function as alternative substrates for the enzymatic reaction and thus dilution of the radioactive taurine; and (c) combination of both mechanisms a and b. Since the procedure for assaying the enzyme reaction relies on the incorporation of radioactive taurine into taurocholate and then solvent extraction of the product in *n*-butanol, there is no simple method to distinguish between either mechanism.

It is demonstrated in Fig. 1 and Table 1 that isethionic acid is the most potent compound that inhibits taurocholate formation (I_{50} = 110 μM). The only structural substitution between isethionic acid and taurine is a hydroxyl moiety in place of the amino group; both compounds are ethanesulfonic acids. However, it had been previously reported from this laboratory [6] that isethionic acid is a substrate for the enzyme system forming cholyl-isethionic acid and thus it is probable that the radioactive taurine is diluted by the alternative, competing substrate. Interestingly, when hypotaurine or glycine was added to the incubation system, extremely high concentrations (7500 and 8800 μM) were necessary to decrease the amount of taurocholate formation by 50 per cent. It has been well documented [4, 5] that glycine and hypotaurine are both substrates for the microsomal conjugating system in rat liver and thus the affinity of glycine and hypotaurine for the enzyme complex must be quite low in the presence of taurine. On the contrary, the affinity of isethionic acid for the enzyme complex must be quite high (whether it is only a substrate or both an inhibitor and a substrate) due to its low I_{50} value (110 μM).

The second most potent inhibitor, aminomethanesulfonic acid (I_{50} = 688 μM), is one carbon shorter than taurine. Whether aminomethanesulfonic acid is a substrate for the enzyme complex or an inhibitor of the enyzmatic reaction has not been determined as yet.

When the formation of taurocholic acid was measured at single concentrations of cholic acid (0.63 mM) and ATP (6.25 mM) but as a function of taurine concentration (Fig. 2), the K_m value for taurine was determined to be 0.18 mM. The inhibitory effects of various concentrations of the taurine analogue, isethionic acid, are shown in Fig. 2. The apparent K_i value for isethionic acid is 61 μM. Similar ex-

Table 1. Inhibition of taurocholate synthesis by structural analogues of taurine*

Compound	Structure	Conc (μM) Required For 50 per cent inhibition
Taurine	$HO_3S—CH_2—CH_2—NH_2$	—
Isethionic acid	$HO_3S—CH_2—CH_2—OH$	110
Aminomethanesulfonic acid	$HO_3S—CH_2—NH_2$	688
Hydroxypropanesulfonic acid	$HO_3S—CH_2—CH_2—CH_2—OH$	3,125
Aminoethanesulfuric acid	$HO_3S—O—CH_2—CH_2—NH_2$	3,750
Hypotaurine	$HO_2S—CH_2—CH_2—NH_2$	7,500†
Glycine	$HOOC—CH_2—NH_2$	8,800†
Cysteine	$HS—CH_2—\underset{\displaystyle COOH}{\underset{\displaystyle \vert}{CH}}—NH_2$	34,400†
Ethanesulfonic acid	$HO_3S—CH_2—CH_3$	43,800†
β-Alanine	$HOOC—CH_2—CH_2—NH_2$	62,000†

* Taurine concentration was kept constant at 130 μM. Inhibitory potency was quantitated by graphically plotting the per cent activity or its reciprocal as a function of the inhibitor concentration.

† These values were determined by extrapolation and thus were not bracketed by experimental observations.

periments were performed utilizing aminomethanesulfonic acid (K_i = 337 μM). Competitive inhibition with respect to taurine was demonstrated for both isethionic acid and aminomethanesulfonic acid.

Hydroxypropanesulfonic acid, the higher homologue of isethionic acid, is only 20 per cent as potent as the parent compound (Table 1; I_{50} = 3125 μM). Thus, the length of the carbon bridge between the amino and sulfonic acid moieties is quite critical.

The sulfuric acid analogue of taurine, aminoethylhydrogensulfate (2-amino-ethanesulfuric acid) is also a relatively weak inhibitor (I_{50} = 3750 μM). The extra length in the molecule produced by the carbon–oxygen–sulfur linkage displaces the amino moiety, and thus the steric configuration of the amino group with respect to the sulfonic acid group is probably not in the correct orientation for a high affinity bond to the enzyme complex.

Cysteine, ethanesulfonic acid and β-alanine are very poor inhibitors (I_{50} values of 34,400, 43,800 and 62,000 μM respectively). Several other compounds, e.g. cysteinesulfinic acid, cysteic acid, aspartic acid and glutamic acid, were also tested but had no effect on the synthesis of taurocholic acid.

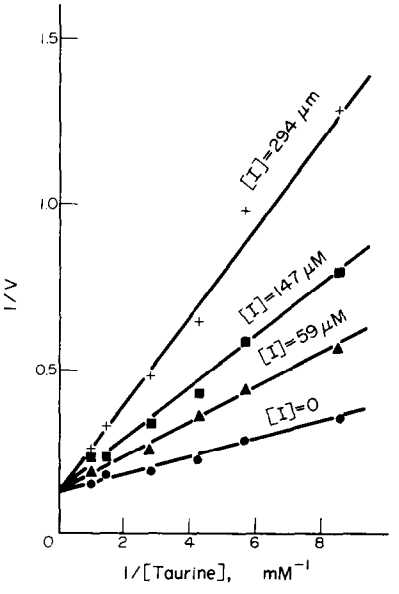

Fig. 2. Lineweaver–Burk plot demonstrating the competitive inhibition of the formation of taurocholic acid by various concentrations of isethionic acid. The reaction system is described in Materials and Methods. The cholic acid concentration was kept constant at 0.63 mM and the ATP concentration was 6.25 mM; taurine concentration varied from 0.12 to 1.00 mM. The incubation was for 30 min at 37°.

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