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Short Communication

STRUCTURE–ACTIVITY RELATIONSHIPS OF ALKYLAMINES THAT INHIBIT RAT LIVER HYDROXYSTEROID SULFOTRANSFERASE ACTIVITIES *IN VITRO*

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Abstract—Tetraalkylammonium salts having *n*-propyl to *n*-amyl side chains inhibited rat liver sulfotransferase (ST) activities toward dehydroepiandrosterone and cortisol, but not ST activity toward 2-naphthol, whereas trialkylamines having ethyl to *n*-amyl side chains inhibited ST activity toward dehydroepiandrosterone, but not ST activities toward cortisol and 2-naphthol. A comparison of I_{50} values, which represent inhibitor concentration resulting in 50% inhibition of dehydroepiandrosterone ST activity, revealed that the values for the tetraalkylammonium salts were 0.015 to 0.017 mM, whereas the values for the trialkylamines were 0.20 to 0.33 mM. Introduction of hydrophilic groups such as hydroxyl, thiol, nitrile and acetamide groups or substitution by methyl and allyl groups in the alkyl side chains markedly diminished the inhibitory effect of triethylamine. These data indicate that ethyl to *n*-amyl side chains are a prerequisite for the alkylamine-type inhibitor. Tertiary amine drugs such as imipramine, dimenhydrinate, cyclizine, chlorpromazine and promethazine inhibited ST activities toward dehydroepiandrosterone and cortisol similar to the tetraalkylammonium salts, although the drugs were weaker inhibitors of hydroxysteroid ST activities. These results imply that in addition to trialkylamine side chains, the other portion of the drugs may participate in the inhibition of hydroxysteroid ST activities.

Key words: hydroxysteroid sulfotransferase; inhibitor; trialkylamine; tetraalkylammonium salt; tertiary amine drug; rat liver

ST† catalyzes the transfer of a sulfate group from PAPS to various acceptor substrates, such as steroids, phenols and amines [1, 2]. Until recently, the multiplicity of ST isoenzymes was based mainly on the purified STs. However, it was difficult to characterize these isoforms on a molecular basis due to their similar catalytic and physical properties [1–3]. Recent cloning studies have provided the basis for characterization of ST isoforms. At present, six rat hydroxysteroid ST cDNAs have been isolated [4–6]. Several investigators purified rat liver steroid ST isoforms, such as hydroxysteroid STs 1, 2 and 3 [1] or glucocorticoid STs I, II and III [7]. Rat liver bile acid ST 1 [8] appears to be hydroxysteroid ST because its *N*-terminal amino acid sequence is highly homologous with that of hydroxysteroid STa [5]. However, a direct comparison has not been made among these purified STs, or between the purified STs and the isolated ST cDNAs. Thus, the number of hydroxysteroid ST isoforms expressed in rat liver is still uncertain.

Special ST inhibitors are important tools for the study of the multiplicity of ST isoforms and for the characterization of the enzyme structure. Recently, we found that trialkylamines having ethyl to *n*-butyl side chains inhibit ST activities toward androsterone and dehydroepiandrosterone, but not ST activities toward cortisol and 2-naphthol [9]. In the present report, we describe the structure–activity relationships of trialkylamines that inhibit dehydroepiandrosterone ST activity and tetraalkylammonium salts that inhibit ST activities toward dehydroepiandrosterone and cortisol. The effects of several drugs with tertiary amine side chains on ST activities are also presented.

Materials and Methods

Materials. [1,2,6,7-^{3}H]Dehydroepiandrosterone (3.7 TBq/mmol) and [4-^{14}C]cortisol (2.0 GBq/mmol) were purchased from New England Nuclear (Boston, MA, U.S.A.). 2-Naphthol and 2-naphthyl sulfate (potassium salt) were from Wako Chemical Ind. (Osaka, Japan) and the Sigma Chemical Co. (St. Louis, MO, U.S.A.), respectively. PAPS was prepared as described by Singer [10]. All other reagents were of the highest grade available.

Animals and preparation of cytosolic fractions. Adult female rats of the Wistar strain were obtained from Sankyo Lab. (Tokyo, Japan). The animals had free access to food and water and were decapitated. A 25% (w/v) liver homogenate was prepared in 0.25 M sucrose and 0.1 M Tris–HCl buffer, pH 7.4. Cytosolic fractions were obtained by differential centrifugation (2000 *g* for 10 min, 16,000 *g* for 45 min, and 105,000 *g* for 60 min).

Enzyme assay. ST activities toward dehydroepiandrosterone, cortisol and 2-naphthol were assayed as described previously [9]. In brief, the incubation medium contained 10 mM $MgCl_2$, 0.1 mM PAPS, 0.1 mM EDTA and 50 μM [^{3}H]dehydroepiandrosterone (0.30 kBq), 40 μM [^{14}C]cortisol (0.22 kBq) or 0.5 mM 2-naphthol, cytosols (50–100 μL) and 0.1 M Tris–HCl buffer, pH 7.4, in the presence of 0–8 mM inhibitor in a total volume of 0.50 mL.

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† Abbreviations: ST, sulfotransferase; and PAPS, 3′-phosphoadenosine 5′-phosphosulfate.

Table 1. Effects of alkylamines and drugs on rat liver ST activities

Inhibitor	Relative ST activity		
	DHEA	CS	2-NAP
Control	1.00 ± 0.10	1.00 ± 0.14	1.00 ± 0.13
Trialkylamine			
Triethylamine	0.43 ± 0.02* (0.23 ± 0.09)	1.02 ± 0.07	0.99 ± 0.04
Diethylmethylamine	0.76 ± 0.03† (0.51 ± 0.20)	1.01 ± 0.03	1.01 ± 0.05
Dimethylethylamine	0.92 ± 0.05	0.99 ± 0.06	1.00 ± 0.05
n-Butyldimethylamine	0.94 ± 0.01	1.02 ± 0.02	1.03 ± 0.08
Diethylallylamine	0.85 ± 0.02 (0.72 ± 0.06)	1.02 ± 0.05	1.00 ± 0.03
2-Diethylaminoethanol	0.90 ± 0.05	1.01 ± 0.02	0.97 ± 0.03
Ethyldiethanolamine	0.98 ± 0.06	1.02 ± 0.02	1.01 ± 0.06
Triethanolamine	0.97 ± 0.06	1.02 ± 0.03	0.99 ± 0.03
Diethylaminoethanethiol	0.81 ± 0.02†	1.03 ± 0.06	1.19 ± 0.16
1-Diethylamino-2-propanol	0.83 ± 0.02†	0.99 ± 0.02	1.03 ± 0.04
3-Diethylamino-1-propanol	0.90 ± 0.03	0.99 ± 0.04	0.98 ± 0.07
Diethylacetamide	1.03 ± 0.03	1.07 ± 0.06	1.03 ± 0.05
Diethylaminoacetonitrile	0.97 ± 0.02	1.03 ± 0.02	1.00 ± 0.06
Tetraalkylammonium chloride			
Tetramethylammonium chloride	0.98 ± 0.04	0.93 ± 0.11	1.04 ± 0.03
Tetraethylammonium chloride	0.38 ± 0.03* (0.15 ± 0.03)	0.96 ± 0.06 (6.53 ± 0.32)	0.99 ± 0.04
Tetra-*n*-propylammonium chloride	0.01 ± 0.01* (0.017 ± 0.002)	0.34 ± 0.02* (0.066 ± 0.004)	1.00 ± 0.03
Tetra-*n*-butylammonium chloride	0* (0.017 ± 0.001)	0.05 ± 0.02* (0.020 ± 0.002)	1.05 ± 0.06
Tetra-*n*-amylammonium chloride	0* (0.015 ± 0.004)	0.02 ± 0.01* (0.019 ± 0.002)	1.07 ± 0.06
Drug			
Imipramine	0.44 ± 0.03*	0.53 ± 0.04*	0.96 ± 0.03
Propranolol	0.54 ± 0.05*	0.90 ± 0.04	ND
Dimenhydrinate	0.57 ± 0.03*	0.68 ± 0.05†	0.84 ± 0.11
Cyclizine	0.62 ± 0.03*	0.57 ± 0.05*	0.97 ± 0.03
Chlorpromazine	0.64 ± 0.01*	0.37 ± 0.05*	0.92 ± 0.01
Promethazine	0.69 ± 0.03*	0.63 ± 0.01†	0.94 ± 0.05
Ritodrine	0.87 ± 0.03	0.84 ± 0.06	0.95 ± 0.03

The concentration of each inhibitor was 0.3 mM, and ST activities were determined as described in Materials and Methods. Each value is the mean ± SD for 3–5 rats. Control ST activities (nmol/min/mg): DHEA, 0.85 ± 0.09; CS, 0.22 ± 0.03; and 2-NAP, 1.01 ± 0.13. ND = not determined. Values in parentheses indicate concentration (mM) of inhibitor resulting in 50% inhibition of control activities (I_{50}). Abbreviations: DHEA, dehydroepiandrosterone; CS, cortisol; and 2-NAP, 2-naphthol.

*,† Relative ST activity was significantly different from the control: *$P < 0.01$, and †$P < 0.05$.

The incubation was carried out at 37° for 10–15 min. The blank values were obtained from control incubations in which PAPS was omitted. The reaction was terminated by heating in a boiling water bath for 1 min. In the assay of dehydroepiandrosterone ST and cortisol ST activities, the reaction mixture was extracted with dichloromethane (3 mL), and a portion (0.3 mL) of the aqueous phase was submitted for the measurement of the radioactivity of the sulfates. In the assay of 2-naphthol ST activity, an aliquot (0.3 mL) of the reaction mixture was mixed with 1.6 M glycine buffer, pH 10.3 (2 mL), and the fluorescent intensity of 2-naphthyl sulfate was measured using excitation and emission wavelengths of 286 and 341 nm, respectively.

Protein concentrations were determined by the method of Bradford [11], with bovine serum albumin as standard.

Results and Discussion

ST activities can be inhibited by substrates and their analogues. Adenosine 3′,5′-bisphosphate, an end product of PAPS, and several ATP analogues, as well as acceptor substrates, are powerful inhibitors of ST isoenzymes [2]. In a previous paper [9], we described that trialkylamines having ethyl to *n*-butyl side chains, which are not substrates for STs, are specific inhibitors of rat liver dehydroepiandrosterone ST activity, whereas trimethylamine is not. In the present study, we first focused our attention on the effects of modification of the alkyl side chains of trialkylamines on ST activities. As shown in Table 1, substitution with methyl or allyl groups markedly diminished the inhibitory effect of trialkylamines at 0.3 mM, the concentration used throughout this study to compare the effects of inhibitors. Furthermore, introduction of hydroxyl, thiol, nitrile and acetamide groups into the alkyl side chains remarkably reduced the inhibitory effect of triethylamine (Table 1). I_{50} values, which represent the inhibitor concentration resulting in 50% inhibition of dehydroepiandrosterone ST activity, demonstrated that diethylmethylamine (I_{50}, 0.51 mM) and diethylallylamine

(0.72 mM) were weaker inhibitors than triethylamine (0.23 mM) (Table 1). In a previous paper [9], we reported that several trialkylamines selectively inhibit dehydroepiandrosterone ST activity in a manner similar to triethylamine. We found that the I_{50} values for tri-*n*-propylamine, tri-*n*-butylamine and tri-*n*-amylamine were 0.20 ± 0.03, 0.26 ± 0.01 and 0.33 ± 0.06 mM, respectively; these values were similar to that of triethylamine (Table 1). These compounds do not inhibit ST activities toward cortisol and 2-naphthol at 0.3 mM [9]. Indeed, 8 mM triethylamine inhibited cortisol ST activity by only 15% (data not shown). It is not known how these trialkylamines can interact with the corresponding ST(s) and why introduction of hydrophilic groups to alkyl side chains or substitution with methyl or allyl groups markedly diminishes the inhibitory effect of trialkylamines.

To the best of our knowledge, the inhibitory effect of tetraalkylammonium salts on ST activities has not been reported. The present study revealed that tetra-*n*-propyl-, tetra-*n*-butyl- and tetra-*n*-amylammonium chlorides strongly inhibit ST activities toward dehydroepiandrosterone and cortisol, but not ST activity toward 2-naphthol (Table 1). It is of interest that tetraethylammonium chloride inhibited dehydroepiandrosterone ST activity, but not cortisol ST activity, whereas tetramethylammonium chloride did not inhibit either of these ST activities (Table 1). With respect to dehydroepiandrosterone ST activity, I_{50} values for the tetraalkylammonium salts were 0.015 to 0.017 mM, whereas the values for the trialkylamines were 0.20 to 0.33 mM. Thus, it becomes evident that the tetraalkylammonium salts are stronger inhibitors of dehydroepiandrosterone ST activity than the trialkylamines and that they also inhibit cortisol ST activity in a similar magnitude. Preliminary kinetic data with partially purified dehydroepiandrosterone ST suggested that inhibition by tetra-*n*-amylammonium salt appears to be a mixed type and is quite different from that of triethylamine, which seems to be a non-competitive inhibitor of both dehydroepiandrosterone and PAPS [9]. Although it is not clear how these amines can interact with the corresponding ST isoenzyme(s), it is expected that these two types of ST inhibitors should provide valuable molecular tools for the study of heterogeneity of hydroxysteroid ST isoenzymes.

It is known that several tertiary amine drugs, such as antidepressants and antihistamines, inhibit human liver ST activities toward dehydroepiandrosterone and estrone [12]. As shown in Table 1, imipramine, dimenhydrinate, cyclizine, chlorpromazine and promethazine inhibited ST activities toward dehydroepiandrosterone and cortisol, but not ST activity toward 2-naphthol. Propranolol, which has a secondary alkylamine side chain, was an inhibitor of dehydroepiandrosterone ST activity, whereas ritodrine, which has a secondary amine side chain, did not inhibit these ST activities significantly (Table 1). Our data appear to be comparable with those of human liver ST activities toward dehydroepiandrosterone and 1-naphthol [12], although those authors used a 1 mM inhibitor concentration and did not determine cortisol ST activity. In humans, chlorpromazine is the most powerful inhibitor of dehydroepiandrosterone ST activity, ritodrine is one of the weakest inhibitors, and the amine drugs are very weak inhibitors of 1-naphthol ST activity [12]. Since amino acid sequences deduced from human and rat dehydroepiandrosterone ST cDNAs or human and rat phenol ST cDNAs exhibit approximately 80 or 90% homology, respectively [3], it is not surprising that these drugs show comparable inhibitory effects on ST activities in these species. It is not clear whether the inhibition arises mainly from their tertiary amine side chain structures. There were some differences in the inhibitory effects between trialkylamines and drugs. Chlorpromazine, promethazine and imipramine have dimethylalkylamine side chains; these structures reduce the inhibitory effect of trialkylamines, as shown in dimethylethylamine and *n*-butyldimethylamine (Table 1). In addition to this, the drugs inhibited ST activities toward dehydroepiandrosterone and cortisol similarly to tetraalkylammonium salts, although their inhibition was much weaker than that of the tetraalkyl compounds. Therefore, in addition to trialkylamine side chains, the other portion of the drugs may participate in the inhibition of these hydroxysteroid ST activities. Thus, further study is needed to clarify the structure–activity relationships of drugs that inhibit hydroxysteroid ST activities. Although the potential for the inhibition of ST activities *in vivo* is difficult to ascertain, these data may suggest possible interaction of these drugs with sulfation of steroid hormones, which may result in disorder of the normal function of steroid hormones.

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