Br. J. clin. Pharmac. (1990), **30**, 127–133

Stereoselective sulphate conjugation of racemic terbutaline by human liver cytosol

T. WALLE & U. K. WALLE
Department of Cell and Molecular Pharmacology and Experimental Therapeutics, Medical University of South Carolina, Charleston, SC, USA

1 The enantioselectivity of the sulphation of racemic terbutaline by phenolsulphotransferases was examined *in vitro* using cytosol from human livers (n = 3) and [^{35}S]-3′-phosphoadenosine-5′-phosphosulphate (PAP^{35}S) as the sulphate donor.
2 The radioactive sulphate conjugate formed was isolated by h.p.l.c. and its enantiomers were separated intact by h.p.l.c. after chiral derivatization.
3 Sulphation of racemic terbutaline occurred with the same apparent K_m value for both enantiomers (270 μM). The extent of sulphation of the (+)-enantiomer was double that of the (−)-enantiomer, solely due to a difference in their apparent V_{max} values.
4 Sulphation of racemic prenalterol, a structural analogue of terbutaline, also showed a two-fold preference for the (+)-enantiomer.
5 These findings suggest that enantioselective sulphate conjugation of chiral phenolic sympathomimetic amine drugs may lead to enantioselective pharmacokinetics that should be considered in the clinical use of these drugs.

Keywords terbutaline prenalterol sulphate conjugation stereoselective sulphation human liver

Introduction

A number of β_2-adrenoceptor agonist drugs are used in the treatment of obstructive lung disease and for the prevention of preterm labour. All of these drugs are phenolic 2-hydroxyethylamines with the hydroxyl group attached to a chiral carbon atom. Most of these drugs are used as racemates, i.e. 50:50 mixtures of the pharmacologically active (−)-enantiomer and the inactive (+)-enantiomer. In two recent clinical studies the pharmacokinetics of the individual enantiomers of one of these drugs, i.e. terbutaline, was shown to be different (Borgström *et al.*, 1989a,b). The oral bioavailability of the (−)-enantiomer was about twice that of the (+)-enantiomer, a difference proposed to be due to enantioselectivity in absorption as well as in first-pass metabolism. As the major metabolic pathway of terbutaline in man is sulphoconjugation (Tegnér *et al.*, 1984), we investigated the stereochemistry of this reaction as a potential contributing factor to this enantioselectivity in pharmacokinetics. Evidence for enantioselectivity in sulphate conjugation of chiral substrates has previously been shown for the propranolol metabolite 4′-hydroxypropranolol in various animal species (Christ & Walle, 1985, 1989; Christ *et al.*, 1990). The rate of sulphation by rat liver cytosol was recently found to be considerably greater for (+)- than for (−)-terbutaline (Walle & Walle, 1989a). However, species differences in the enantioselectivity of sulphate conjugation of chiral substrates may be considerable (Christ & Walle, 1985).

In the present study we examined the enantioselective sulphoconjugation of racemic terbutaline using human liver cytosol as the source of phenolsulphotransferases and the co-substrate 3′-phosphoadenosine-5′-phosphosulphate (PAPS)

Correspondence: Dr Thomas Walle, Department of Cell and Molecular Pharmacology and Experimental Therapeutics, Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29425, USA

as the sulphate donor. We also examined the enantioselective sulphation of the racemic form of the structural analogue prenalterol, which also undergoes sulphation as the main metabolic pathway in man (Hoffmann *et al.*, 1982).

Methods

Materials

(±)-Terbutaline sulphate salt, (+)- and (−)-terbutaline hydrobromide, and (±)-terbutaline sulphate ester were kindly donated by AB Draco (Lund, Sweden). Prenalterol ((−)-4-[2-hydroxy-3-(2-propylamino)propoxy]phenol) hydrochloride, H80/62 base ((±)-prenalterol), and (±)-prenalterol sulphate ester were generously provided by AB Hässle (Mölndal, Sweden). [^{35}S]-3′-Phosphoadenosine-5′-phosphosulphate (PAP^{35}S) (specific activity 1.0–1.5 Ci mmol^{-1}) was purchased from New England Nuclear (Wilmington, DE, USA). 2,6-Dichloro-4-nitrophenol (DCNP) and *p*-nitrophenol were obtained from Aldrich (Milwaukee, WI, USA). 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) was synthesized as described previously (Walle *et al.*, 1985).

Liver cytosol

Histologically normal human liver samples were obtained through the Liver Tissue Procurement and Distribution System (LTPADS, University of Minnesota, Minneapolis, MN, USA). Tissue was obtained from a 51-year-old man, a 54-year old woman (Female 1) and a 16-year-old woman (Female 2). The cause of death was cerebral haemorrhage or gunshot headwound. The livers were removed shortly after circulatory arrest, cut into small pieces and stored at −80° C. Liver cytosol was prepared from 4–8 g of tissue according to Sekura & Jakoby (1979) by ultracentrifugation of a 25% (w/v) homogenate in 10 mM Tris-HCl (pH 7.4) with 0.25 M sucrose and 3 mM 2-mercaptoethanol. All steps were carried out at 0–4° C. The cytosol was stable for several months when stored at −80° C. Protein content was assayed according to Lowry *et al.* (1951).

Incubation conditions

The incubations were carried out essentially according to Anderson & Weinshilboum (1980), i.e. with 0.4 μM PAP^{35}S as cosubstrate in pH 7.4 33 mM Tris-HCl, containing bovine serum albumin (BSA, 0.0625%), dithiothreitol (8 mM), 0.5–2 μl cytosol (10–40 μg cytosolic protein per 310 μl incubate) and varying final concentrations of (±)-terbutaline (25–500 μM for each enantiomer) or (±)-prenalterol (0.5–500 μM) as the substrate. These assay conditions were chosen as they have been used in studies involving a variety of human tissues and substrates (Anderson & Weinshilboum, 1980; Campbell *et al.*, 1987a,b; Reiter *et al.*, 1983; Sundaram *et al.*, 1989; Young *et al.*, 1984). It must, however, be pointed out that the PAPS concentration in this assay is nonsaturating. Thus, although the apparent K_m values are valid at any PAPS concentration, the apparent V_{max} values can only be compared to results obtained with an identical PAPS concentration. After 30 min incubation at 37° C and the addition of about 10 μg of synthetic unlabelled racemic sulphate conjugate (Walle & Walle, 1989a), protein and PAPS were precipitated with barium hydroxide and zinc sulphate (Foldes & Meek, 1973).

The effect of DCNP and elevated temperature on terbutaline sulphate formation was tested using 500 μM (±)-terbutaline as the substrate and 40 μg protein per incubate. Preincubations with DCNP (5 μl, 0–10 μM, in acetone) were for 20 min at 37° C. In experiments testing the effect of elevated temperature on the sulphation reaction, cytosol was diluted with an equal volume of Tris buffer (no BSA or dithiothreitol). Aliquots were heated in a water bath at 43° C for 0 to 30 min, cooled on ice, and used in 30 min, 37° C terbutaline incubations. The pH optimum of the reaction was explored using sodium acetate buffer (33 mM) at pH 5.0–7.0 and Tris-HCl buffer (33 mM) at pH 7.5–9.5.

Analysis of the enantiomers of intact sulphate conjugates

After incubation and barium hydroxide/zinc sulphate precipitation, the radiolabelled sulphate conjugates were isolated from the supernatant (400 μl injected of a total volume of 710 μl) by reversed-phase h.p.l.c. U.v. detection at 280 nm was made possible by the added unlabelled synthetic racemic sulphates. Terbutaline sulphate was isolated using a Spherisorb ODS-1 column, 5 μm particle size, 25 cm × 4.6 mm (Alltech, Deerfield, IL, USA), and a mobile phase of methanol-water-acetic acid (13:86:1, v/v) in 0.05 M ammonium acetate (pH 4.0) at a flow rate of 1 ml min^{-1}. The eluent associated with the terbutaline sulphate peak was collected, freeze-dried and derivatized with 30 μl 0.03 M GITC in 0.2% v/v triethylamine in acetonitrile–water (75:25, v/v) (Walle *et al.*, 1985). The GITC diastereomers of the intact sulphate conjugate (15 μl aliquots) were separated on the same

column, with a mobile phase of acetonitrile–methanol–water–acetic acid (30:5:64:1, v/v) in the same buffer as above at 1 ml min^{-1}. The individual diastereomer peaks were collected and their radioactivity, from ^{35}S-labelled conjugates formed during the incubation, counted by liquid scintillation spectrometry. The order of elution of the diastereomers was determined from incubates with the enantiomerically pure (+)- and (−)-terbutaline substrates.

The procedure was identical when racemic prenalterol was used as the substrate. The retention times for racemic prenalterol sulphate and the corresponding GITC diastereomers were slightly longer than for terbutaline sulphate.

The sulphation of *p*-nitrophenol was examined in an identical fashion to that of Campbell *et al.* (1987b), using a simple nonchromatographic assay.

Data generated were in general based on the mean value of duplicate incubates. Differences in the formation of the (+)- and (−)-enantiomers were evaluated using Student's paired *t*-test with a significance level of $P < 0.05$.

Results

Each of the three human livers investigated was examined with respect to the sulphation of the commonly used substrate *p*-nitrophenol, which has been shown to produce a peak enzyme activity at about 4 μM and an apparent K_m of about 1 μM in a previous study using human liver cytosol (Campbell *et al.*, 1987b). Our results were virtually identical, including the maximum velocities (in counts min^{-1}) for the sulphation of this substrate.

In our study of both racemic terbutaline and prenalterol, counts obtained for each enantiomeric pair either without substrate, or cytosol, or PAP^{35}S or using boiled cytosol were identical to scintillation cocktail blanks. The validity of the assay was tested by spiking the cytosol with purified, enzymatically generated ^{35}S-labelled terbutaline sulphate. The recovery of the individual enantiomers after chiral derivatization was 82 ± 5% (mean ± s.e. mean). The minimum detectable concentration was 20 pmol l^{-1} and the interassay coefficient of variation, using 250 μM (+)- or (−)-terbutaline as substrate was 8%.

Effect of pH, cytosolic protein and incubation time

Racemic terbutaline sulphation (500 μM, of each enantiomer) demonstrated no activity at pH 5.5 and then a slow increase in activity from pH 6.0 to a maximum at pH 9.0. The sulphation of (+)-terbutaline was favoured over that of (−)-terbutaline by about 1.8-fold over this range. The enantioselectivity was thus independent of pH. All kinetic determinations were made at pH 7.4, where the activity was about 70% of maximum. At this pH, the formation of both (+)- and (−)-terbutaline sulphate from racemic terbutaline was linear at cytosolic protein concentrations up to 40 μg and for incubation times up to 40 min. Similar results were obtained when racemic prenalterol was used as the substrate.

Kinetics of sulphation

The sulphation of racemic terbutaline was studied at substrate concentrations ranging from 0.5 to 500 μM of each enantiomer, using 10 μg of cytosolic protein and a 30 min incubation time. The lowest concentration at which activity could be measured was 25 μM. Mean values for the velocity (counts min^{-1}) *vs* substrate concentration (μM) in three livers are shown in Figure 1a. Typical hyperbolic curves for a single reaction were obtained for both enantiomers with the rate of sulphate conjugation favouring the (+)-enantiomer by about 1.9-fold. The corresponding double reciprocal plots are shown in Figure 1b with correlation coefficients of 0.997. The apparent enzyme kinetic parameters calculated from these plots for the three individual livers are shown in Table 1. The apparent K_m values for the (+)- and (−)-terbutaline of about 270 μM are the same. The observed enantioselective sulphate conjugation of racemic terbutaline is thus due to a 1.9-fold greater velocity in the formation of (+)-terbutaline sulphate. The values for both K_m and velocity in the individual livers were similar, with the single male liver producing data in between those of the two female livers.

For racemic prenalterol typical hyperbolic curves for a single reaction were obtained for both enantiomers from 2.5 to 100 μM with the rate of conjugation favouring the (+)-enantiomer by about 1.8-fold. At higher substrate concentrations the velocity of the reaction showed a significant decrease. The apparent enzyme kinetics calculated for substrate concentrations up to 100 μM demonstrated an apparent K_m of 34 ± 6 μM (mean ± s.e. mean; $n = 3$) for (−)-prenalterol and 31 ± 8 μM for (+)-prenalterol with the enantioselectivity entirely due to a 1.8-fold greater velocity in the formation of (+)-prenalterol sulphate than of (−)-prenalterol sulphate. The velocity was about three-fold higher for prenalterol than for terbutaline. As with terbutaline, the sulphation of prenalterol varied little among the three livers.

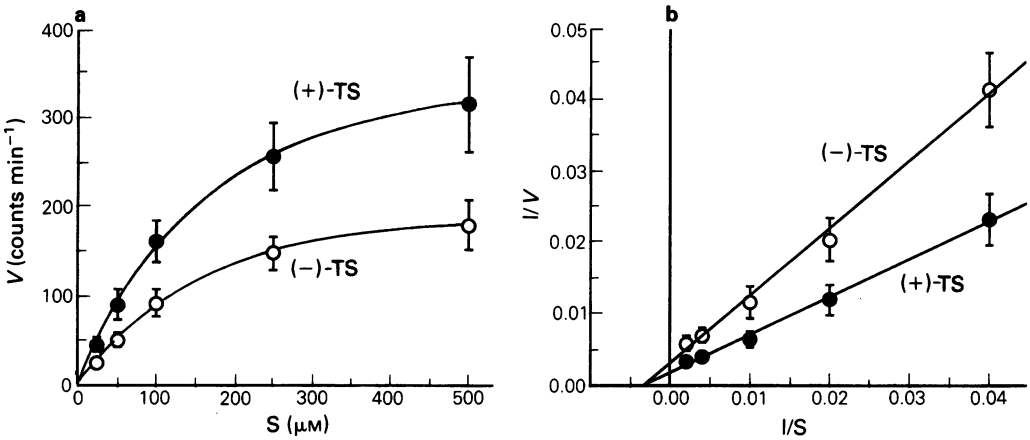

Figure 1 Enantioselective sulphation of racemic terbutaline by human liver cytosol using ^{35}S-labelled PAPS as cosubstrate. a) Velocity *vs* substrate concentration; b) The corresponding double reciprocal plots. Mean values ± s.e. from three livers are shown. (−)-TS, (−)-terbutaline sulphate; (+)-TS, (+)-terbutaline sulphate.

Table 1 Apparent enzyme kinetic parameters describing the sulphoconjugation of racemic terbutaline [(±)-T] by human liver cytosol

	K_m (μM)			V_{max} (pmol min^{-1} mg^{-1} protein)		
Liver	*(+)-T*	*(−)-T*	*(+)-T/(−)-T*	*(+)-T*	*(−)-T*	*(+)-T/(−)-T*
Male	253	247	1.02	0.627	0.351	1.79
Female 1	198	202	0.98	0.968	0.470	2.06
Female 2	370	343	1.08	0.567	0.312	1.82
Mean	274	264	1.03	0.721	0.378[a]	1.89
s.e. mean	51	42	0.03	0.125	0.048	0.09

[a]Significantly lower than the value for (+)-terbutaline; $P < 0.05$.

Effect of DCNP and temperature on the sulphation of terbutaline

As inhibition of sulphation by dichloronitrophenol (DCNP) and temperature was previously shown to be useful in characterizing human phenol-sulphotransferase activities (Rein *et al.*, 1982; Reiter & Weinshilboum, 1982; Reiter *et al.*, 1983), this was examined for the sulphation of racemic terbutaline (250 μM of each enantiomer) using 40 μg of cytosolic protein. DCNP inhibited the sulphation of both terbutaline enantiomers to the same extent (Figure 2a). The inhibition curves gave an IC_{50} of 5 μM. Preincubation of the cytosol at 43° C for various times also produced a parallel inhibition of the sulphation of both terbutaline enantiomers (Figure 2b) with a 50% inhibition after 20 min.

Discussion

This study has demonstrated that the sulphate conjugation of racemic terbutaline by human liver cytosol is stereoselective, favouring the (+)-enantiomer over the (−)-enantiomer by about two-fold. This observation, which represents the first demonstration of enantioselective sulphation of a chiral drug by a human tissue, may contribute to the enantioselective oral bioavailability of this drug in man. In addition, we also demonstrated a similar enantioselectivity in the sulphate conjugation of the structural analogue prenalterol. Although the number of livers studied was small, all showed very similar stereoselectivity.

The higher oral bioavailability of (−)- than of (+)-terbutaline in man (Borgström *et al.*, 1989 a,b) was suggested to depend both on incomplete

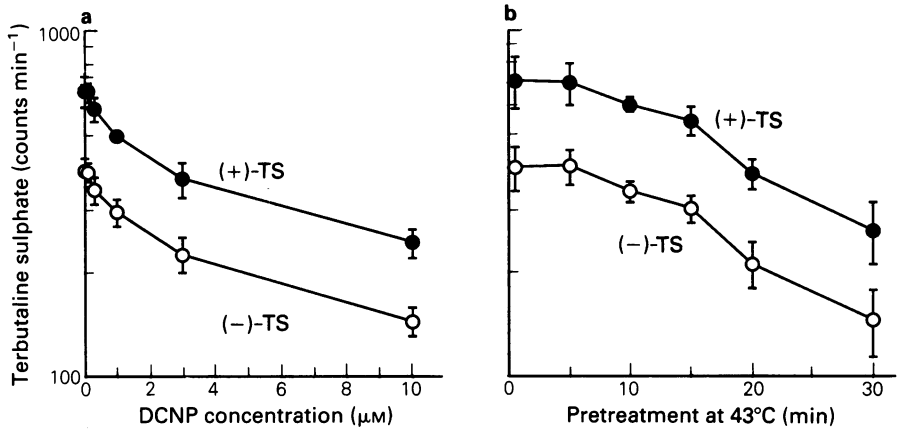

Figure 2 Effects of a) dichloronitrophenol (DCNP) and b) time (preincubation at 43° C) on the enantioselective sulphation of racemic terbutaline by human liver cytosol. The concentration of terbutaline was 250 μM of each enantiomer. Mean values ± s.e. mean using three livers are shown. (−)-TS, (−)-terbutaline sulphate; (+)-TS, (+)-terbutaline sulphate.

absorption of the (+)-enantiomer and on a more efficient presystemic metabolism of this enantiomer. As sulphate conjugation is the main pathway in the metabolic clearance of terbutaline in man (Tegnér *et al.*, 1984), our findings *in vitro* of preferential sulphation of (+)-terbutaline may thus explain the enantioselective presystemic metabolism. The site of presystemic metabolism of terbutaline has been claimed to be the gut wall, not the liver (Borgström *et al.*, 1989a; Nyberg, 1984). However, although the phenolsulphotransferase composition in the gut wall is somewhat different from that in the liver, the isoenzymes in the two tissues appear to be the same (Sundaram *et al.*, 1989). Another factor to consider is the unknown contribution of glucuronidation to the clearance of terbutaline (Tegnér *et al.*, 1984), a metabolic pathway that may have a different enantioselectivity from that of sulphation. A third factor is the so called 'deficit' in the disposition of terbutaline (Borgström *et al.*, 1989b), known to be about 20% of the dose after i.v. doses, but unknown after oral doses, which may include biliary-faecal elimination of conjugates with unknown stereochemical composition. Additional *in vitro* and *in vivo* studies will thus be of importance in order to assess the significance of our current *in vitro* observations.

The apparent K_m for the sulphation of racemic terbutaline was identical for the two enantiomers, i.e. about 270 μM. The enantioselectivity was entirely due to a two-fold higher apparent V_{max} for the (+)-enantiomer. Attempts were made to characterize the phenolsulphotransferase(s) involved in this reaction, based on inhibition by DCNP and elevated temperature, as described previously for the sulphation of achiral substrates (Rein *et al.*, 1982; Reiter & Weinshilboum, 1982; Reiter *et al.*, 1983). These authors classify the phenolsulphotransferase enzymes as phenol (P) forms, which are selectively inhibited by low concentrations of DCNP or pentachlorophenol, and monoamine (M) forms, which are selectively inhibited by pretreatment at elevated temperature. Both treatments were inhibitory, suggesting that terbutaline sulphation is mediated by both P and M forms of the soluble phenolsulphotransferases. The stereochemical composition of the terbutaline sulphates formed was unaffected by either treatment, implying similar stereoselectivity for both enzyme forms. In contrast, in a preliminary communication the sulphation of 4′-hydroxypropranolol by the same human liver cytosol showed two distinct enzyme activities, one being stereoselective and involving the M form and the other being nonstereoselective and involving the P form (Walle & Walle, 1989b).

Using rat liver cytosol we found an eight-fold enantiomeric preference for the sulphation of (+)- compared with (−)-terbutaline, due to a difference in apparent V_{max} values (Walle & Walle, 1989a). The apparent K_m values in the rat, about 1200 μM for both enantiomers, were, however, very high. Similar species differences in apparent enzyme kinetics have been observed for a number of achiral substrates using rat and human phenolsulphotransferases (Campbell *et al.*, 1987a). Moreover, as shown *in vivo*, in the rat the sulphation of terbutaline is an inefficient process compared with glucuronidation (Conway *et al.*, 1973; Singhvi *et al.*, 1974; Tegnér *et al.*,

1984). This species is thus a poor model of man for the clearance of terbutaline.

The enantioselectivity for the sulphation of the structural analogue prenalterol was almost identical to that of terbutaline, and was also entirely dependent on a difference in apparent V_{max} values. The configuration around the chiral carbon atom is identical for these two molecules but with only one phenolic substituent for prenalterol. The eight-fold lower apparent K_m for prenalterol (about 33 μM) compared with terbutaline may be a reflection of the greater lipophilicity of prenalterol. This is further emphasized by data for 4'-hydroxypropranolol, the lipophilic naphthyl analogue of prenalterol, which has an apparent K_m of only 1 μM (Walle & Walle, 1989b). It should be pointed out that racemic prenalterol was studied as a model compound; in clinical use the pure (−)-enantiomer is employed.

The observations of stereoselective *in vitro* sulphate conjugation of both terbutaline and prenalterol serve to emphasize that this is also likely to occur with a number of other chiral phenolic sympathomimetic amine drugs, which also undergo sulphation in man, including salbutamol (Morgan *et al.*, 1986), isoprenaline (Causon *et al.*, 1984), metaproterenol (Macgregor *et al.*, 1983), xamoterol (Marten *et al.*, 1984), ritodrine (Brashear *et al.*, 1988), and fenoterol (Rominger & Pollmann, 1972; Sodha & Schneider, 1984). Of these drugs enantioselective pharmacokinetics of the parent drug have been shown only for salbutamol, based on urinary data (Tan & Soldin, 1987), suggesting that the rate of sulphoconjugation may be considerably higher for the (−)- than for the (+)-enantiomer, i.e. in contrast to our findings with terbutaline and prenalterol. Further evaluation of the pharmacokinetics of the individual enantiomers of this important class of drugs is needed with respect to enantioselectivity in their sulphate conjugation.

This study was supported in part by the NIH Grants HL 29566 and GM 41141. We are indebted to Lars Borgström of AB Draco and K.-J. Hoffmann of AB Hässle for the provision of the terbutaline and prenalterol compounds, respectively.

References

Anderson, R. J. & Weinshilboum, R. M. (1980). Phenolsulphotransferase in human tissue: Radiochemical enzymatic assay and biochemical properties. *Clin. Chim. Acta*, **103**, 79–90.

Borgström, L., Chang-Xiao, L. & Walhagen, A. (1989a). Pharmacokinetics of the enantiomers of terbutaline after repeated oral dosing with racemic terbutaline. *Chirality*, **1**, 174–177.

Borgström, L., Nyberg, L., Jönsson, S., Lindberg, C. & Paulson, J. (1989b). Pharmacokinetic evaluation in man of terbutaline given as separate enantiomers and as the racemate. *Br. J. clin. Pharmac.*, **27**, 49–56.

Brashear, W. T., Kuhnert, B. R. & Wei, R. (1988). Maternal and neonatal urinary excretion of sulfate and glucuronide ritodrine conjugates. *Clin. Pharmac. Ther.*, **44**, 634–641.

Campbell, N. R. C., Van Loon, J. A., Sundaram, R. S., Ames, M. M., Hansch, C. & Weinshilboum, R. (1987a). Human and rat liver phenol sulfotransferase: Structure-activity relationships for phenolic substrates. *Mol. Pharmac.*, **32**, 813–819.

Campbell, N. R. C., Van Loon, J. A. & Weinshilboum, R. M. (1987b). Human liver phenol sulfotransferase: Assay conditions, biochemical properties and partial purification of isozymes of the thermostable form. *Biochem. Pharmac.*, **36**, 1435–1446.

Causon, R. C., Desjardins, R., Brown, M. J. & Davies, D. S. (1984). Determination of *d*-isoproterenol sulphate by high-performance liquid chromatography with amperometric detection. *J. Chromatogr.*, **306**, 257–268.

Christ, D. D. & Walle, T. (1985). Stereoselective sulfate conjugation of 4-hydroxypropranolol *in vitro* by different species. *Drug Metab. Dispos.*, **13**, 380–381.

Christ, D. D. & Walle, T. (1989). Stereoselective sulfation of R,S-4'-hydroxypropranolol by canine hepatic cytosol and partially purified phenol-sulfotransferases. *J. Pharmac. exp. Ther.*, **251**, 949–955.

Christ, D. D., Walle, U. K., Oatis, J. E., Jr. & Walle, T. (1990). Pharmacokinetics and metabolism of the pharmacologically active 4'-hydroxylated metabolite of propranolol in the dog. *Drug Metab. Dispos.*, **18**, 1–4.

Conway, W. D., Singhvi, S. M., Gibaldi, M. & Boyes, R. N. (1973). The effect of route of administration on the metabolic fate of terbutaline in the rat. *Xenobiotica*, **3**, 813–821.

Foldes, A. & Meek, J. L. (1973). Rat brain phenol-sulfotransferase – partial purification and some properties. *Biochim. Biophys. Acta*, **327**, 365–374.

Hoffmann, K.-J., Arfwidsson, A. & Borg, K. O. (1982). The metabolic disposition of the selective β_1-adrenoceptor agonist prenalterol in mice, rats, dogs, and humans. *Drug Metab. Dispos.*, **10**, 173–179.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem.*, **193**, 265–275.

Macgregor, T. R., Nastasi, L., Farina, P. R. & Keirns, J. J. (1983). Isolation and characterization of

metaproterenol-3-O-sulfate: A conjugate of metaproterenol in human urine. *Drug Metab. Dispos.*, **11**, 568–573.

Marten, T. R., Bourne, G. R., Miles, G. S., Shuker, B., Rankine, H. D. & Dutka, V. N. (1984). The metabolism of ICI 118,587, a partial agonist of β_1-adrenoceptors, in mice, rats, rabbits, dogs, and humans. *Drug Metab. Dispos.*, **12**, 652–660.

Morgan, D. J., Paull, J. D., Richmond, B. H., Wilson-Evered, E. & Ziccone, S. P. (1986). Pharmacokinetics of intravenous and oral salbutamol and its sulphate conjugate. *Br. J. clin. Pharmac.*, **22**, 587–593.

Nyberg, L. (1984). Pharmacokinetic parameters of terbutaline in healthy man. An overview. *Eur. J. resp. Dis.*, **65** (Suppl. 134), 149–160.

Rein, G., Glover, V. & Sandler, M. (1982). Multiple forms of phenolsulphotransferase in human tissues: Selective inhibition by dichloronitrophenol. *Biochem. Pharmac.*, **31**, 1893–1897.

Reiter, C. & Weinshilboum, R. M. (1982). Acetaminophen and phenol: Substrates for both a thermostable and a thermolabile form of human platelet phenol sulfotransferase. *J. Pharmac. exp. Ther.*, **221**, 43–51.

Reiter, C., Mwaluko, G., Dunnette, J., Van Loon, J. & Weinshilboum, R. (1983). Thermolabile and thermostable human platelet phenol sulfotransferase: Substrate specificity and physical separation. *Naunyn-Schmiedeberg's Arch. Pharmac.*, **324**, 140–147.

Rominger, K. L. & Pollmann, W. (1972). Vergleichende Pharmakokinetik von Fenoterol-Hydrobromid bei Ratte, Hund und Mensch. *Arzneimittel-Forsch.*, **22**, 1190–1196.

Sekura, R. D. & Jakoby, W. B. (1979). Phenol sulfotransferases. *J. biol. Chem.*, **254**, 5658–5663.

Singhvi, S. M., Conway, W. D., Gibaldi, M. & Boyes, R. N. (1974). Influence of dose on the metabolism and excretion of terbutaline in the rat. *Xenobiotica*, **4**, 563–570.

Sodha, R. J. & Schneider, H. (1984). Sulphate conjugation of β_2-adrenoceptor stimulating drugs by platelet and placental phenol sulphotransferase. *Br. J. clin. Pharmac.*, **17**, 106–108.

Sundaram, R. S., Szumlanski, C., Otterness, D., Van Loon, J. A. & Weinshilboum, R. M. (1989). Human intestinal phenol sulfotransferase: Assay conditions, activity levels and partial purification of the thermolabile form. *Drug Metab. Dispos.*, **17**, 255–264.

Tan, Y. K. & Soldin, S. J. (1987). Analysis of salbutamol enantiomers in human urine by chiral high-performance liquid chromatography and preliminary studies related to the stereoselective disposition kinetics in man. *J. Chromatogr.*, **422**, 187–195.

Tegnér, K., Nilsson, H. T., Persson, C. G. A., Persson, K. & Ryrfeldt, Å. (1984). Elimination pathways of terbutaline. *Eur. J. resp. Dis.*, **65** (Suppl. 134), 93–100.

Walle, T., Christ, D. D., Walle, U. K. & Wilson, M. J. (1985). Separation of the enantiomers of intact sulfate conjugates of adrenergic drugs by high-performance liquid chromatography after chiral derivatization. *J. Chromatogr.*, **341**, 213–216.

Walle, U. K. & Walle, T. (1989a). Stereoselective sulfation of terbutaline by the rat liver cytosol: Evaluation of experimental approaches. *Chirality*, **1**, 121–126.

Walle, U. K. & Walle, T. (1989b). Stereoselective sulfate conjugation of chiral phenolic amine drugs by human liver cytosol. *Eur J. clin. Pharmac.*, **36** (Suppl.), A 180.

Young, W. F., Okazaki, H., Laws, E. R., Jr. & Weinshilboum, R. M. (1984). Human brain phenol sulfotransferase: Biochemical properties and regional localization. *J. Neurochem.*, **43**, 706–715.

(Received 23 October 1989, accepted 13 March 1990)