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COMMON FOOD ADDITIVES ARE POTENT INHIBITORS OF HUMAN LIVER 17α-ETHINYLOESTRADIOL AND DOPAMINE SULPHOTRANSFERASES

KATHLEEN J. BAMFORTH, ALWEN L. JONES, RICHARD C. ROBERTS* and MICHAEL W. H. COUGHTRIE†

Departments of Biochemical Medicine and *Medicine, University of Dundee, Ninewells Hospital and Medical School, Dundee DD1 9SY, U.K.

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Abstract—Interactions between dietary xenobiotics, drugs and biologically active endogenous compounds are a potential source of idiosyncratic adverse pathology. We have examined the inhibition of the sulphation of a number of xenobiotics and endobiotics in human liver cytosol by 15 food additives and constituents. Sulphation of dehydroepiandrosterone was resistant to inhibition by all compounds tested; however, dopamine sulphotransferase (ST) activity was inhibited strongly by (±)-catechin, (+)-catechin, octyl gallate, tartrazine and vanillin. Sulphation of the xenobiotic steroid 17α-ethinyloestradiol (EE_2) was inhibited by vanillin, erythrosin B and octyl gallate. Of these compounds, only vanillin was found to be sulphated to a significant extent by both human liver and platelets, and vanillin was determined to be a substrate for the monoamine-sulphating isoenzyme of phenolsulphotransferase. Vanillin was found to inhibit 50% of liver EE_2 ST activity (IC_{50}) at a concentration of approximately 1.3 μM and the mode of inhibition was non-competitive. The implications of these results for the adverse side effects associated with food additives and oral contraceptives are discussed.

Many of the compounds with which we are constantly challenged are without nutritive value, material which nevertheless is ingested, inhaled or absorbed [1]. The enzymes of detoxification provide the means for inactivation, transport and excretion of these xenobiotics and are therefore a critical part of the body's defence against such potentially harmful compounds. Conjugation with sulphate is an important pathway for the *in vivo* metabolism and inactivation of a host of xenobiotics and endogenous compounds, including steroid hormones, bile salts and monoamine neurotransmitters [2–4]. As a result of the addition of a sulphate group from the donor molecule 3′-phosphoadenosine 5′-phosphosulphate (PAPS‡), the biological activity of the parent compound is in general decreased, the water solubility increased and excretion facilitated. These reactions are carried out by a family of sulphotransferases (STs), cytosolic enzymes found in most body tissues, including liver, intestine, brain, adrenals and platelets [3, 5]. In common with other detoxication enzyme systems, the STs exist as a multi-gene family, and in man a number of subfamilies have been identified based on protein purification studies [e.g. 6–8] and more recently cDNA cloning [e.g. 9–11]. Hydroxysteroid ST (HST) is responsible for the sulphation of steroids (principally androgens) and bile acids, and phenol STs (PSTs), sulphate simple phenols (P-PST) and phenolic monoamines (M-PST). Isolation and heterologous expression of one form of human HST and one form of P-PST have recently been reported [9–11]. Controversy still exists over the identity of the human ST isoenzyme(s) responsible for the sulphation of oestrogens. In rats, a distinct oestrogen ST has been purified [12–14] and cloned [13] (E. B. Borthwick, A. Burchell and M. W. H. Coughtrie, unpublished), which bears substantial amino acid sequence homology to rat phenol ST. However, in man no such distinct protein has been isolated to date. Anion exchange chromatography of human liver cytosol and subsequent assay of resolved fractions for oestrogen ST activity with oestrone and 17β-oestradiol suggested that oestrone was principally sulphated by HST, and 17β-oestradiol was a substrate for P-PST [15]. However, recent (unpublished) work from this laboratory has demonstrated the presence of a novel ST in human liver, immunochemically related to the PST subfamily, which sulphates the synthetic oestrogen 17α-ethinyloestradiol (EE_2), as well as oestrone (K. J. Bamforth and M. W. H. Coughtrie, manuscript in preparation), and a ST sulphating oestrone has been isolated from human foetal liver [16].

Several natural and synthetic food and drink constituents have been shown to be potent and specific inhibitors of PST [17]. Inhibition of sulphotransferase activity *in vivo* by such compounds as food additives, colourants and preservatives is likely to result in disruption of the sulphation of

† Corresponding author: Dr Michael W. H. Coughtrie, University Department of Biochemical Medicine, Ninewells Hospital and Medical School, Dundee DD1 9SY, U.K. Tel. (0382) 632510; FAX (0382) 644620.

‡ Abbreviations: ST, sulphotransferase; EE_2, 17α-ethinyloestradiol; PAPS, 3′-phosphoadenosine 5′-phosphosulphate; PST, phenolsulphotransferase; P-PST, phenol-sulphating phenolsulphotransferase; M-PST, monoamine-sulphating phenolsulphotransferase; HST, hydroxysteroid sulphotransferase; DHEA, dehydroepiandrosterone; FPLC, fast protein liquid chromatography.

both endogenous compounds and co-administered xenobiotics (e.g. drugs) and therefore may pose clinical problems. Studies from this laboratory have shown that a number of drugs are also potent inhibitors of human liver steroid sulphation *in vitro* [18].

EE_2 is now more or less exclusively used as the oestrogenic component of combined oral contraceptive preparations. In the liver it is metabolized principally by the cytochrome P450 monooxygenase system [19]; however, conjugation with glucuronic acid and more importantly sulphate predominate in the gastrointestinal mucosa, contributing to the significant first-pass metabolism of EE_2 [20]. EE_2 represents a particularly interesting ST substrate to study, firstly since it is both a xenobiotic and a steroid, and secondly the role of EE_2 in the generation of side effects associated with combined preparation oral contraceptive use and the consequences of interaction with other co-administered xenobiotics has been the source of much debate [e.g. 21, 22]. Inhibition of the sulphation of EE_2 in the gastrointestinal tract by dietary chemicals may have a significant impact on the bioavailability of EE_2 and therefore on the susceptibility to adverse side effects associated with the use of this drug. We have therefore investigated the interaction between numerous food additives and the sulphation of a number of endogenous and xenobiotic ST substrates, with particular emphasis on dopamine and EE_2. Our results show that commonly used food additives, in particular vanillin which is widely used in flavouring ice cream, yoghurt and confectionery, are potent inhibitors of human liver ST activity, and in particular of the sulphation of EE_2 and dopamine. These results have important implications for our understanding of the pathogenesis of adverse effects associated with the use of EE_2-containing oral contraceptive preparations and with over-exposure to food additives.

MATERIALS AND METHODS

Chemicals. 1-[1-^{14}C]Naphthol (58 mCi/mmol) and ECL western blotting detection reagents were purchased from Amersham International (Aylesbury, U.K.). [1,2,6,7-^{3}H]Dehydroepiandrosterone (100 Ci/mmol), [2,4,6,7-^{3}H(N)]oestrone (91 Ci/mmol), 17α-[6,7-^{3}H(N)]ethinyloestradiol (40–60 Ci/mmol) and 3′-phosphoadenosine 5′-phospho-[^{35}S]-sulphate (PAP[^{35}S]S) (2.54 Ci/mmol) were from Dupont/New England Nuclear (Stevenage, U.K.). Oestrone, dehydroepiandrosterone (DHEA), EE_2, dopamine, 5-hydroxytryptamine, barium acetate, barium hydroxide, zinc sulphate, ecteola cellulose and peroxidase-conjugated anti-(rabbit IgG), adsorbed with human serum proteins, were obtained from the Sigma Chemical Co. (Poole, U.K.). 1-Naphthol and phenol were purchased from Merck (Glasgow, U.K.). Aspartame, protocatechuic acid, (±)-catechin, saccharin, benzoic acid, gallic acid, *p*-hydroxybenzoic acid, tannic acid, (+)-catechin, tartrazine, 4-chlorobenzoic acid, vanillin, octyl gallate, erythrosin B and propyl gallate were obtained from Aldrich (Poole, U.K.). PAPS, fast protein liquid chromatography (FPLC) instrumentation and columns were purchased from Pharmacia (Milton Keynes, U.K.). Scintillation fluid (Emulsifier Safe) was purchased from Canberra Packard (Pangbourne, U.K.). All other reagents were obtained from commonly used local suppliers, and were of at least analytical grade.

Liver samples, and preparation of cytosols. Human liver samples (50–500 g, histologically normal) were obtained from patients undergoing liver resection surgery or from organ transplant donors, and were immediately frozen in liquid nitrogen and stored at −70°. Cytosols were harvested by differential centrifugation from 20% homogenates prepared in 10 mM triethanolamine/HCl, 10% glycerol, 5 mM 2-mercaptoethanol, pH 7.4. Homogenates were centrifuged at 10,000 *g* for 10 min and the resulting supernatants centrifuged at 105,000 *g* for 1 hr. The supernatants were aspirated, carefully avoiding the lipid layer at the surface, aliquoted and stored at −70° until use (within 2 months of preparation).

Platelet preparation. From healthy adult volunteers 50 mL of blood were collected by venepuncture into 1 mL of 5% disodium EDTA and mixed gently. Samples were kept at room temperature and platelets processed within 2 hr. The samples were centrifuged at 300 *g* for 10 min, and the platelet-rich plasma carefully aspirated and re-centrifuged at 4000 *g* for 20 min. The platelet pellet obtained was washed and resuspended in 5 mM potassium phosphate, pH 7.5 prior to homogenization. The homogenates were stored at −70° until assayed (within 2 months). For FPLC, platelet cytosol was used, obtained by centrifugation of the homogenate for 45 min at 105,000 *g*. Protein content of platelet homogenates was determined as for liver cytosol. Ethical approval was obtained from the Ethics Committee of Tayside Health Board. Only subjects expressing substantial levels of platelet M-PST and P-PST were employed in this study.

Sulphotransferase enzyme assays. Sulphation of 1-naphthol, DHEA, oestrone and EE_2 was determined *in vitro* by sensitive radiometric assays using labelled substrates which we have modified and adapted from published methods. The conditions for quantitation of the sulphation of 1-naphthol, DHEA and oestrone have been recorded previously [17]. To assay EE_2 ST activity the substrate and cosubstrate (PAPS) concentration, pH, incubation time and cytosolic protein content were optimized and employed throughout. Briefly, EE_2 ST activity was measured in 10 μL of dilute cytosol where EE_2 and PAPS were present at concentrations of 0.33 and 0.5 μM, respectively, in a total assay volume of 150 μL. The buffer comprised 200 mM Tris–HCl pH 8.5, 1 mM $MgCl_2$. This mixture was incubated for 45 min at 37° and the reaction terminated by the addition of 3 mL chloroform followed by 0.25 mL 0.25 M Tris–HCl pH 8.7. Following a single extraction, an aliquot (0.2 mL) of the aqueous phase was subjected to liquid scintillation counting in 3 mL Emulsifier Safe.

For the inhibition experiments, 10 μL of inhibitor, dissolved in water, were added to the assays. Control incubations containing 10 μL of water were set up in parallel.

To assay ST activity towards all other substrates, a modification of the radioenzymatic method of

Foldes and Meeks [23], with PAP[^{35}S]S as sulphate donor, was used. All assays were carried out in duplicate and the incubation mixture consisted of 20 μg cytosolic protein and potassium phosphate buffer (10 mM, pH 7.4) in a volume of 110 μL and either 20 μL substrate solution or 20 μL water (for control incubations). Reactions were initiated by the addition of 20 μL PAP[^{35}S]S. Following incubation for 10 min at 37° the reactions were terminated by the addition of 0.2 mL of 0.1 M barium acetate. Unreacted PAPS was removed by precipitation with 0.2 mL of 0.1 M barium hydroxide and 0.2 mL of 0.1 M zinc sulphate. After centrifugation 0.5 mL of the supernatant was removed and, after addition of 4 mL scintillant and thorough mixing, radioactivity was quantitated by liquid scintillation spectrometry. Assay of ST activity towards acidic substrates was also carried out using the ecteola cellulose procedure described by Whittemore and Roth [24], a modification of the method of Borchardt *et al.* [25], which is claimed to be more suitable for use with acids [26]. All sulphotransferase assays were performed in duplicate.

FPLC analysis of human platelet cytosol sulphotransferase. Platelets were prepared as described except that they were resuspended in 20 mM triethanolamine, 3 mM mercaptoethanol, pH 7.4 (FPLC buffer). All solutions were made using double-distilled deionized water and were filtered through a 0.22 μm filter prior to use. The MonoQ anion exchange column was first washed and equilibrated with FPLC buffer prior to loading 4 mg of total platelet cytosol protein, and all steps were performed at a flow rate of 1 mL/min. The column was washed with 10 mL of FPLC buffer followed by 10 mL of FPLC buffer containing 100 mM NaCl. A linear gradient of 100–250 mM NaCl in FPLC buffer was generated and 1 mL fractions were collected and immediately placed on ice. The total protein content of individual fractions was estimated by continuous monitoring of the absorbance at 280 nm of the eluate. These fractions were then assayed as described for ST activities.

Gel electrophoresis and immunoblot analysis. Samples were resolved on 11% polyacrylamide (monomer) gels in the presence of 0.1% SDS, according to the method of Laemmli [27]. For immunoblot analysis, proteins resolved by SDS–PAGE were electrophoretically transferred to nitrocellulose (Schleicher and Schuell, BA 85) [28], and chemiluminescent detection of immunoreactive polypeptides was performed using the ECL western blotting detection system as described by the manufacturer (Amersham). The primary antibody used was a rabbit anti-(rat liver oestrone ST) which has previously been shown to detect human platelet M- and P-PST isoenzymes on immunoblot analysis [14].

Protein estimation. The protein content of cytosol preparations was estimated using the method of Lowry *et al.* [29] with bovine serum albumin (fraction V, Boehringer Mannheim, Lewes, U.K.) as standard, on samples which had been thawed only once.

RESULTS

We have investigated the effects of a wide range of commonly used food additives (e.g. colourants, preservatives, flavourings) on a number of different ST enzyme activities in human liver cytosol *in vitro*. Although liver cytosol was used to investigate the effects of dietary xenobiotics on sulphotransferase activity, there is to date no firm evidence that the

Table 1. Inhibition of human liver STs by food additives

Compound	% Inhibition of ST activity				
	EE_2	Dopamine	1-Naphthol	DHEA	Oestrone
(±)-Catechin	31 ± 10	84 ± 5	23 ± 9	12	67
(+)-Catechin	34	85	4	—	75
4-Chlorobenzoic acid	—	9 ± 9	18 ± 9	—	41
Aspartame	—	52 ± 6	14 ± 10	6	27
Benzoic acid	19 ± 5	53 ± 10	5 ± 11	—	12
Erythrosin B	77 ± 5	18 ± 22	14 ± 10	31	16
Gallic acid	43 ± 16	54 ± 15	20 ± 9	31	18
Octyl gallate	62 ± 4	68 ± 12	44 ± 10	7	20
p-Hydroxybenzoic acid	12 ± 16	47 ± 14	17 ± 1	2	54
Propyl gallate	46 ± 6	59 ± 26	26 ± 10	5	20
Protocatechuic acid	—	56 ± 14	15 ± 9	6	40
Saccharin	—	44 ± 7	13 ± 11	8	—
Tannic acid	43 ± 15	54 ± 16	26 ± 10	—	61
Tartrazine	17 ± 5	94 ± 3	14 ± 12	12	60
Vanillin	72 ± 4	100 ± 0	91 ± 3	4	85

Data are expressed as mean % inhibition ± SEM for duplicate determinations made on four different human liver samples (DHEA and oestrone ST assays were performed on two cytosols—values quoted are means) and are derived from the enzyme activity in cytosol assay in the presence of the inhibitor (at a concentration of 6.7 μM) compared to the control activity (water substituted for inhibitor).

— No inhibition observed with this compound.

sulphotransferases present in different tissues possess different physical or catalytic properties.

Initial screening of the effects of 15 compounds on the sulphation of DHEA, oestrone, 1-naphthol dopamine and EE_2 demonstrated that three of these (vanillin, octyl gallate and erythrosin B) were strong inhibitors (>60%) of hepatic EE_2 ST activity at a concentration of 6.7 μM (Table 1). When the effect of octyl gallate on the inhibition of other ST activities was examined, this compound was found to show weaker inhibition (15–40%) of oestrone and 1-naphthol ST activity and essentially no inhibition (<10%) of DHEA ST activity. Similarly, the pattern of inhibition with erythrosin B showed that the oestrone, 1-naphthol and DHEA ST activities were again only moderately inhibited (15–40%) in comparison to the effect on EE_2 ST activity. The results obtained with the natural flavouring compound vanillin were particularly interesting. As stated, this resulted in strong (>60%) inhibition of EE_2 ST activity and vanillin was also a very good inhibitor of dopamine, oestrone and 1-naphthol ST activities, however inhibition of the DHEA ST was negligible (<4%). The DHEA ST activity was refractory to inhibition by all of the compounds tested, a particularly significant observation in view of the current debate regarding the identity of the human ST isoenzyme(s) responsible for the sulphation of oestrogens [15, 18]. This differential inhibition of DHEA ST over oestrogen and phenol ST activities was also seen with a variety of drugs [18], strongly supporting the view that oestrone and EE_2 are not significant substrates for the DHEA ST isoenzyme. Dopamine ST was particularly sensitive to inhibition by a number of these compounds, suggesting that they may be able to interfere with the sulphation of substrates for the M-PST isoenzyme, including dopamine and 5-hydroxytryptamine. The implications for this in modulating levels of monoamine neurotransmitters in the brain is likely to be determined by the ability of the compounds to cross the blood–brain barrier.

In order to determine whether these chemicals were substrates for sulphation, we assayed human platelet homogenate and liver cytosol for ST activity towards a number of them using the PAP[^{35}S]S assay methods described by Foldes and Meek [23] and Whittemore and Roth [24]. The use of the ecteola cellulose method [24] is supposedly more suitable for the assay of acidic substrates [26] and this method was therefore employed in addition to the barium precipitation method of Foldes and Meek [23]. The results in Table 2 show that of the compounds tested, only vanillin was found to be a significant substrate for sulphation in both human platelets and liver. Aspartame, *p*-hydroxybenzoic acid, propyl gallate, gallic acid and tannic acid were also sulphated, but generally at extremely low rates indicating that the capacity of humans for the sulphation of these compounds is insignificant. To identify the ST isoenzyme responsible for the sulphation of vanillin in human platelets, anion exchange FPLC of human platelet cytosol was performed on a MonoQ column. Assay of the resulting fractions demonstrated that vanillin ST activity co-eluted with the M-PST activities towards dopamine and 5-hydroxytryptamine, and was not associated with the P-PST activity towards phenol (Fig. 1A), indicating that the M-PST form is responsible for the sulphation of vanillin. In this analysis, two peaks of M-PST activity were resolved, each possessing the ability to sulphate all three M-PST substrates (dopamine, 5-hydroxytryptamine and vanillin), although there appeared to be more activity associated with the peak eluting at lower salt concentration (Fig. 1A). The data in Fig. 1A were confirmed when selected chromatography fractions were subjected to immunoblot analysis with an anti-(rat liver oestrogen ST) antibody preparation which cross-reacts with human platelet M- and P-PST isoenzymes [14]. (Fig. 1B). Both peaks of M-PST enzyme activity corresponded to the presence of an immunoreactive polypeptide of relative molecular mass 34 kDa. The observation that vanillin is sulphated by M-PST is consistent with the approximately 10-fold higher rates of sulphation (V_{max} platelets = 30 pmol/min/mg, liver = 3.8 pmol/min/mg; K_m platelets = 4 μM, liver 40 μM—not shown) in platelets than liver, resulting from the higher level of expression of M-PST in platelets compared to liver [6].

In an attempt to determine the nature of the inhibition of EE_2 ST activity by vanillin, kinetic analysis was performed. Firstly, the IC_{50} value (the concentration resulting in 50% inhibition of EE_2 ST activity) for vanillin was estimated (Fig. 2). From this analysis it was determined that a concentration of 1.3 μM vanillin resulted in approximately 50% inhibition of EE_2 ST activity. Using vanillin at this concentration, its effect on the kinetic parameters of EE_2 sulphation in two different human liver cytosols was examined. The data obtained (Table 3) suggest that the mechanism of inhibition of EE_2 ST activity by vanillin was non-competitive in nature, with the K_m remaining constant while the V_{max} was decreased in the presence of vanillin.

DISCUSSION

Our ever-increasing demand for "convenience" foods is highly dependent on the use of a large number of chemicals as flavourings, colourants and preservatives. It is therefore of considerable importance to understand the consequences of the interactions between these chemicals and (a) other xenobiotics to which we are exposed and (b) important endogenous compounds such as steroid hormones and neurotransmitters. Through an appreciation of such interactions will come further understanding of the aetiology of the many idiosyncratic adverse responses to xenobiotics.

The potential role of food additives in food intolerance has received particular attention and, in recent years, unfavourable publicity. Widespread concern about their safety has been expressed as increasingly these compounds are associated with such conditions as hyperactivity in children, asthma, allergic reactions and general ill health [e.g. 30]. Children appear particularly susceptible to adverse effects from food additives, many manifesting as behavioural problems, and this is thought to be as a result of the consumption of large amounts of confectionery, food and beverages containing high

Table 2. Assay of human liver cytosol and platelet homogenate for ability to sulphate food additives

Compound	Platelets: barium precipitation	Liver: barium precipitation	Platelets: ecteola cellulose	Liver: ecteola cellulose
Vanillin	27	2.7	29	2.0
(+)-Catechin	ND	ND	2.5	ND
p-Hydroxybenzoic acid	ND	0.9	ND	0.6
Aspartame	ND	ND	5.0	ND
Protocatechuic acid	ND	ND	ND	ND
Gallic acid	ND	0.3	0.9	ND
Tannic acid	ND	0.6	ND	1.0
Propyl gallate	ND	ND	1.9	0.3
Octyl gallate	ND	ND	ND	ND

Enzyme activities are expressed as pmol/min/mg protein, and were determined using PAP[^{35}S]S in human liver cytosol and platelet homogenates by the barium precipitation [23] or ecteola cellulose [24] methods.

ND, Activity below the limit of sensitivity of the assay method (approx. 0.3 pmol/min/mg under the conditions employed).

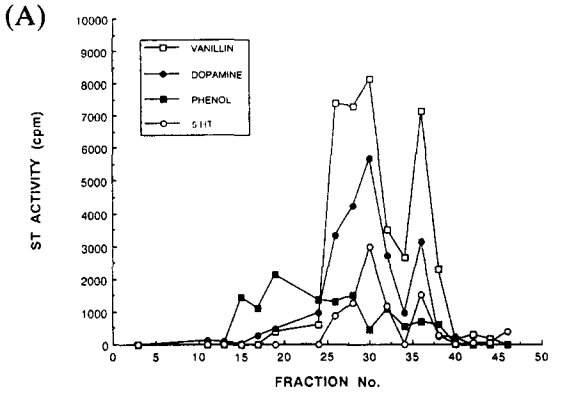

Fig. 1. Anion exchange FPLC of human platelet cytosol and analysis of resolved ST activities. (A) Platelet cytosol was chromatographed as described in Materials and Methods. Fractions (1 mL) were assayed for sulphotransferase activity towards the following substrates: vanillin (□), dopamine (●), phenol (■) and 5-hydroxytryptamine (○). (B) Protein in selected fractions from separation represented in A (67 μL) and 100 μg human platelet cytosol (P) were resolved on an SDS–polyacrylamide gel (11% acrylamide monomer) and electroblotted onto nitrocellulose. Following exposure to anti-(rat liver oestrone ST) at a concentration of 2.25 μg/mL, immunoreactive polypeptides were detected using peroxidase conjugated anti-(rabbit IgG) with chemiluminescence detection. The arrows indicate the mobilities of M-PST (34 kDa) and P-PST (32 kDa), respectively.

concentrations of artificial colourings and flavourings [e.g. 31]. The mechanisms of such behavioural side effects resulting from over-exposure to food additives are as yet unclear, but for example erythrosin B, a fluorescein dye used to colour food and drinks, was found to be a potent non-competitive inhibitor of dopamine uptake *in vitro* by nerve endings prepared from rat brain [32], suggesting that interference with dopaminergic function in the brain by such compounds may be involved. The present work (Table 1) demonstrated that tartrazine, a yellow/orange dye widely used as a food colourant (until

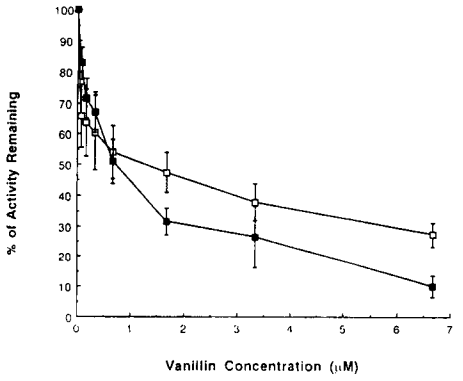

Fig. 2. Determination of the IC_{50} for the inhibition of EE_2 and 1-naphthol ST activity in human liver cytosol. EE_2 and 1-naphthol ST activities were assayed in duplicate on four different human liver cytosols in the presence of vanillin at concentrations between 0.06 and 6.7 μM. Data points are means ± SEM.

Table 3. Kinetic analysis of human liver EE_2 ST activity following inhibition by vanillin

	Control	+ Vanillin
Liver cytosol 1 K_m	5.7	5.9
V_{max}	42.4	15.8
Liver cytosol 2 K_m	6.9	4.3
V_{max}	34.5	8.0

Data were obtained from Lineweaver–Burk plots (by computer linear regression analysis) derived from duplicate determinations on two different human liver cytosol preparations, in the presence or absence of 1.3 μM vanillin. Control incubations contained water in place of vanillin.

recently when concern over its safety has led to a gradual decline in its use) was a potent inhibitor of dopamine ST. Also vanillin, used as a flavouring in confectionery and ice cream, completely inhibited the sulphation of dopamine (Table 1) and subsequent analysis determined that this compound was, like dopamine, a substrate for both of the M-PST forms of phenolsulphotransferase resolved on our FPLC analysis (Fig. 1). Reduced metabolism of bioactive compounds such as dopamine following inhibition of M-PST by dietary compounds may result in an increased bioavailability and the consequent altered pharmacology. This may also be of importance in migraine, where many sufferers report that their attacks can be precipitated by a wide variety of foods and beverages ("dietary migraine"). The results presented here are particularly interesting in the light of recent focus on the role of 5-hydroxytryptamine regulation of intracranial blood vessel diameter in the generation of migraine [33, 34] and the development of the 5-hydroxytryptamine$_1$-like receptor agonist sumatriptan as a novel and effective treatment for migraine [34, 35]. 5-Hydroxytryptamine is a substrate for both of the M-PST isoenzymes (Fig. 1), and therefore dietary compounds which interfere with its metabolism may be involved at some point in the pathogenesis of dietary migraine. The observation that vanillin, a xenobiotic, is a substrate for M-PST is also of interest since most substrates for this isoenzyme are usually important endogenous phenolic or catechol monoamines such as dopamine, 5-hydroxytryptamine and other neurotransmitters [5]. While this manuscript was being completed, a report appeared indicating that vanillin was a potent inhibitor of rat liver aryl sulphotransferase activities towards both phenol and dopamine, and suggesting that the mode of inhibition was consistent with a partial non-competitive or mixed type model [36]. These authors also noted that vanillin was a substrate for sulphotransferase in rat liver, however they did not identify for which isoenzyme(s), although another recent publication from the same laboratory [37] demonstrated that a cloned rat liver phenol ST expressed in COS-7 cells was able to sulphate phenol, dopamine and vanillin.

As EE_2 is the major oestrogenic component of the vast majority of combined oral contraceptive preparations, it ranks as one of the world's most widely used drugs. It is absorbed totally from the gastrointestinal tract and peak concentrations are observed in the plasma 1–2 hr after oral ingestion; however, it has a bioavailability of only about 40%, indicative of an extensive first-pass metabolism. Indeed, rapid conjugation occurs in the intestinal wall producing principally the conjugate EE_2 sulphate, but also some EE_2 glucuronide [10]. These conjugates are then excreted in bile and can subsequently undergo hydrolysis by gut flora to produce free EE_2 which is reabsorbed, thereby generating enterohepatic circulation [38]. Should dietary compounds such as those found here to be *in vitro* inhibitors of EE_2 (vanillin, octyl gallate, erythrosin B) be able to inhibit the sulphation of EE_2 in the intestinal mucosa *in vivo*, a reduction in the first-pass metabolism of the drug would result, which would increase systemic bioavailability of EE_2 resulting in concentrations of EE_2 higher than are required to provide effective contraception. More emphasis would then be put on the other principal route of EE_2 metabolism, 2-hydroxylation by cytochromes P450 IIIA4 and members of the IIC family [19, 39]. The potential increased synthesis of reactive catechol oestrogens from EE_2 as a result of inhibition of conjugative metabolism by food additives is clearly a potential mechanism for the generation of adverse side effects. The identity of the human sulphotransferase responsible for the sulphation of EE_2 has not been reported to date. However, recently we have identified what appears to be a new form of ST of molecular mass 33 kDa in human liver cytosol which sulphates EE_2 and which is recognised by the anti-(rat liver oestrone ST) used in this work (Fig. 2), but which is not P-PST (32 kDa), M-PST (34 kDa) nor HST (35 kDa) (K. J. Bamforth and M. W. H. Coughtrie, manuscript in preparation). Further characterization of this EE_2 ST will aid our understanding of the role of sulphation in EE_2 metabolism and toxicity.

There has been much debate about the role of

EE_2 in the side effects associated with combined oral contraceptive therapy, and current evidence suggests that while EE_2 may play a role in the appearance of various tumours associated with oral contraceptive use, it may also be beneficial through protecting women against certain types of cancer [21]. It has however been observed that high EE_2 concentrations can result in the formation of EE_2–protein conjugates *in vivo*, possibly as a result of the formation of reactive catechol oestrogens, which lead to the generation of anti-EE_2 antibodies [40]. High levels of circulating immune complexes in some women have been associated with increased incidence of vascular disease [41].

We have shown that certain widely used food additives are potent inhibitors ($IC_{50} \cong 1\ \mu M$ for vanillin) of the sulphation of EE_2 by human liver cytosol. These data suggest that interactions between dietary xenobiotics and EE_2 may play a role in the generation of the side effects associated with the use of EE_2. Additionally, inhibition of M-PST enzyme activity (involved in the inactivation of neurotransmitters including dopamine and 5-hydroxytryptamine) by food additives was observed, suggesting a possible mechanism for the production of the side effects (e.g. behavioural) associated with excess exposure to food additives. It is of course necessary to establish the occurrence of these inhibitions *in vivo* but the results presented here contributed to our knowledge and understanding of the interactions between dietary xenobiotics, important endogenous compounds and drugs.

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