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Minireview

Sulfoconjugation of exo- and endogenous phenols: species and tissue specificity

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Biotransformation of exo- and endogenous compounds includes the phase I and phase II reactions [1]. The products of phase I: oxido-reduction and hydrolysis, serve as substrates for the phase II enzymes, which conjugate the functional groups with polar endogenous compounds, such as sulfate, glucuronic acid, glutathione, amino acids [2]. The conjugation converts lipid soluble substances into polar molecules, more easily excreted *via* kidney [3]. Also this process decreases harmful physiological, pharmacological or toxic properties of conjugated compounds.

Conjugation with sulfate is an important pathway for biotransformation of steroid hormones, bile acids, neurotransmitters and various xenobiotics [4 - 6]. More than five decades ago Richter observed that up to 70% of an orally administered dose of epinephrine was excreted in the urine as a pharmacologically inactive sulfate ester [7]. The primary role of sulfation in catecholamine inactivation was later confirmed by other researchers [8, 9]. Johnson *et al.* [10] have shown that over 99% of total dopamine and almost 80% of total norepinephrine and epinephrine exist in human blood plasma as sulfate esters. Abnormal levels of dopamine and other biogenic amines are implicated in the etiology of Parkinson's disease, schizophrenia, depression, migraine [11 - 14]. Sulfation of catecholamines plays an important role in regulation of blood pressure and in the treatment of blood pressure disorders [15, 16].

D-Cysteine as a precursor of sulfate

Sulfoconjugation requires inorganic sulfate which, under normal conditions in mammals, is chiefly derived from the oxidation of L-cysteine which is a precursor of cosubstrates for several conjugations. D-Cysteine, the unphysiological isomer, is not utilized for protein, glutathione or taurine synthesis, therefore we have tested it as a selective precursor for inorganic sulfate, required for sulfation of xenobiotics [17]. In the experiments *in vivo* we found that, in rats, the rate of sulfoxidation is very similar for L- and D-isomers, thus stereospecificity for an amino acid seems to play no role. Since the administration of L- or D-cysteine yielded a similar increase in sulfation, D-cysteine can be used to enhance selectively sulfate availability [17]. Inorganic sulfate, transferred to 3'-phosphoadenosine-5'-phosphosulfate (PAPS), becomes a physiological donor of the sulfate group [18].

Phenol sulfotransferase (PST[1], EC 2.8.2.1), a cytosolic enzyme, transfers the sulfate group from PAPS to various endo- and exogenous phenolic compounds including catecholamines and drugs [5, 19]. Since sulfation is a detoxication phase II reaction, phenol sulfotransferase has been studied primarily in liver [20 - 24]. Later reports on PSTs from other tis-

[1]Abbreviations: COMT, catechol-*O*-methyltransferase; MAO, monoamino oxidase; MD, α-methyldopa; PAPS, 3'-phosphoadenosine-5'-phosphosulphate; PST, phenol sulfotransferase; TL, thermolabile.

sues, in particular from brain and platelet were published [25 - 29].

In our research started in 1980 we have studied properties of phenol sulfotransferases from different mammalian tissues: those directly exposed to exogenous substances (lung, gastrointestinal tract), very sensitive to catecholamines (brain) and exogenous phenolic compounds (reproductive organs), as well as those responsible for excretion of sulfate esters (liver, kidney). Some observations were also made with the isolated (enterocytes) or cultured cells (Caco-2 from human colon adenocarcinoma, endothelial cells from bovine and monkey brain microvessels).

Sulfation in lung

Lungs represent the first line of defence with respect to compounds entering the body *via* the respiratory tract or transported to lungs *via* pulmonary artery [30].

Studies of Gibby *et al.* [31, 32] with short-term organ cultures of normal human peripheral lung and with tumour tissue from the same patient, showed striking qualitative differences in conjugation of 1-naphthol, a model phenolic substrate. Tumour tissues formed almost exclusively the glucuronic acid conjugates with barely detectable amounts of sulfates, whereas in the normal lung tissue mainly sulfate ester conjugates were found.

Our comparative studies of highly purified phenol sulfotransferases from normal human and bovine lung demonstrated significant species differences in their properties [33 - 35].

The human lung PST reacts with catecholamines and exogenous phenols, whereas the bovine enzyme only with phenols (Table 1). Molecular weight of human lung phenol sulfotransferase is 35000, which corresponds to the M_r of the subunit of bovine lung enzyme [33, 35]. Since the M_r of the native bovine lung PST is 69000, this means that a lung PST is a dimer, similarly as the rat liver enzyme [35].

The human lung PST is thermostable when assayed with low concentrations of phenol, but thermolabile with dopamine and phenol at high concentration. In contrast, the bovine enzyme is thermostable at all phenol concentrations [34].

At varying concentrations of phenol the human and bovine lung enzymes obey the non -Michaelis-Menten kinetics [34]. The plot consists of two phases, at higher and at lower concentrations, suggesting either the presence of different molecular forms, as it has been demonstrated for human platelet and brain enzymes [28, 36], or the presence of more than one active centre for the substrate. The high affinity of human lung PST towards catecholamines shows that sulfation is an important reaction for detoxication of those compounds,

Table 1

Activity of human and bovine lung phenol sulfotransferases with different substrates [34]

Substrate (30 μM)	Human lung PST activity (%)	Bovine lung PST activity (%)
2-Naphthol	100	100
p-Nitrophenol	100	96
Phenol	70	48
Salicylamide	56	45
p-Methylphenol	72	90
o-Methylphenol	340	105
Epinephrine	250	0
Norepinephrine	28	0
Dopamine	500	0
DOPA	0	0
Tyrosine	0	0

while a lack of PST activity with catecholamines in bovine lung indicates that, in this species, they are metabolized by a different pathway.

Sulfation in gastrointestinal tract

The gastrointestinal tract is the first line of defence against noxious substances administered orally or present in the diet. The ability of the small intestinal epithelium to control the exchange of substances between the intestinal tract and the systemic circulation makes it a biological barrier to drugs and nutrient absorption. According to Powell *et al.* [37] a normal human diet may contain up to 600 mg of phenolic compounds daily. In portal blood only conjugated phenol is present, mainly (72%) as phenyl sulfate.

Differences in substrate specificity and thermostability of phenol sulfotransferases isolated from human ileum enterocytes and from bovine small intestine [38, 35] are the same as those of the enzyme from lung of those species [33, 35]. These results are consistent with the results of Sundaram *et al.* [39] on the human jejunum enzyme. However, although the kinetic plot of human ileum PST determined with phenol consists of two phases, like that of lung PST, the bovine intestine enzyme is characterized by Michaelis-Menten kinetics.

Extending the studies on substrate specificity of phenol sulfotransferases from small intestine of other species we have found that rat and guinea pig enzymes, similarly as bovine phenol sulfotransferase, are active with *p*-nitrophenol but not with dopamine, whereas the dog enzyme reacts with both substrates (unpublished). Highly purified PST from rat stomach mucosa sulfates mainly exogenous phenolic compounds and neutral metabolites of catecholamines, but not catecholamines themselves [40].

Although the potential of the so-called "gut-wall" metabolism in absorption of exogenous substances is recognized, the characteristics of the enzymes mediating those biotransformations have not been investigated in detail probably due to the lack of an adequate experimental model system.

Since attempts to culture enterocytes or to establish cell lines derived from the small intestine have been unsuccessful [41, 42], we have used the cell line Caco-2.

Caco-2 cells derive from human colon carcinoma which undergoes enterocytic differentiation in culture [43]. This differentiation involves not only development of morphological features such as tight junctions and microvilli, but also the expression of the enterocytic enzymes [44, 45]. Caco-2 cells have been proposed as a transport model system of the small intestinal epithelium [45].

PST isolated from Caco-2 cells cultured for different periods of time exhibit properties similar to those of the human small intestine enzyme in that both show activity with dopamine and *p*-nitrophenol [38, 45]. The specific activity of Caco-2 PST with either substrate increases with age of the cells and so does its thermal stability [46] which, unlike that of human small intestine PST, is not associated with the acceptor substrate used. Despite the differences in thermal stability, the Caco-2 cell PST shows numerous similarities with PST from the intestinal enterocytes, thus the cells can be used in the study of sulfation.

Sulfation in brain

Catecholamines are inactivated in the central nervous system by several enzymatic modifications including deamination, *O*-methylation, and *O*-sulfation. In human brain the affinity of PST towards catecholamines is much higher than that of monoamino oxidase (MAO) A and B, or cytosolic catechol-*O*-methyltransferase (COMT), but similar to that of the membrane-bound COMT [47].

PST is widely distributed throughout the brain, and in human brain it shows the highest activity in the cortex [36]. The affinity of the human brain enzyme for dopamine is about a 100 times as high as that of PSTs in most laboratory animals [48]. Only the African green monkey brain PST sulfates dopamine with similar affinity as does the human enzyme. Since the only species suitable as a model for sulfate conjugation in man appeared to be the African green monkey [46], in our research we have included PST from the brain of *Rh. macaca* monkey [27, 49 - 52].

Unlike the bovine brain cortex which contains only one PST form, that of the monkey contains two [49, 52]. The main form in monkey brain cortex is the anionic one corresponding to about 90% of the total activity. This form, similarly as the bovine brain enzyme, is a dimer of

M_r about 66000, whereas the cationic PST is an active monomer of about 35000.

Bovine PST, similarly as the cationic monkey brain form, is active with exogenous phenolic compounds, and with deaminated, or both deaminated and methylated metabolites of catecholamines, but not with parent catecholamines (Figs. 1, 2 A). Catecholamines which are not sulfated by those two enzymes are substrates for the anionic monkey form (Fig. 2 B) as well as for TL (thermolabile) or M (monoamine) PST which according to Young *et al.* [36] is one of two, and according to Whittemore *et al.* [53] one of three molecular forms present in human brain. The anionic *Rhesus macaca* PST, like the human brain M PST or the African green monkey enzyme, shows very high affinity towards dopamine [49, 48, 53].

Catecholamines, their metabolites and exogenous phenols are also sulfated by PST from both rat brain gray matter and astrocytes (unpublished).

All the enzymes studied with several endogenous substrates, showed the highest activity with 3-methoxy-4-hydroxyethylene glycol, the major norepinephrine metabolite in the brain [50, 52].

6-Hydroxydopamine, a neurotoxin that selectively destroys catecholaminegic neurons [54] is sulfated (inactivated) by both monkey brain PSTs (Fig. 2), but not by the bovine enzyme (Fig. 1 A).

Since the involvement of PST in brain metabolism of drugs has not been studied in detail, we investigated some of those compounds as substrates or inhibitors for both monkey and bovine enzymes [50 - 52]. Among sympathicomimetics, octopamine, phenylephrine and metaraminol are sulfated by the anionic monkey PST (Fig. 3 B), whereas only phenylephrine by the cationic form (Fig. 3 A). The drugs lacking a single free benzyl hydroxyl group such as mephentermine, ephedrine, pseudoephedrine and methoxamine are neither substrates nor inhibitors for either monkey PST [50]. None of those drugs is sulfated by bovine brain enzyme [52].

α-Methyldopa (MD), a methylated precursor of catecholamines that is widely used as an antihypertensive drug, as well as its metabolites: normetanephrine and metanephrine are also sulfated both by the monkey brain and bovine PSTs (Figs. 3, 4). Although the affinity of monkey brain enzymes for MD is low, sulfation can effectively influence the therapeutic effect of this drug, since the anionic PST shows a high affinity towards normetanephrine which is thought to play an important role in the antihypertensive action of MD acting as a false neurotransmitter.

Minoxidil, an antihypertensive drug used often after MD treatment, is pharmacologically active as the *N,O*-sulfate ester [55]. This compound is sulfated (activated) by both monkey brain PSTs (Fig. 3), [50, 51].

Sulfation in blood-brain barrier

The brain microvessel endothelial cells form the so-called "blood-brain barrier" [56, 57]. These cells are bound by tight, intercellular junctions and have few pinocytic vesicles. Plasma membranes from endothelial cells and the basement membrane are highly lipophilic, therefore nonpolar molecules can penetrate into the brain, whereas the transport of most of the polar compounds is restricted. However, several transcellular transport systems do exist at the blood-brain barrier to provide the central

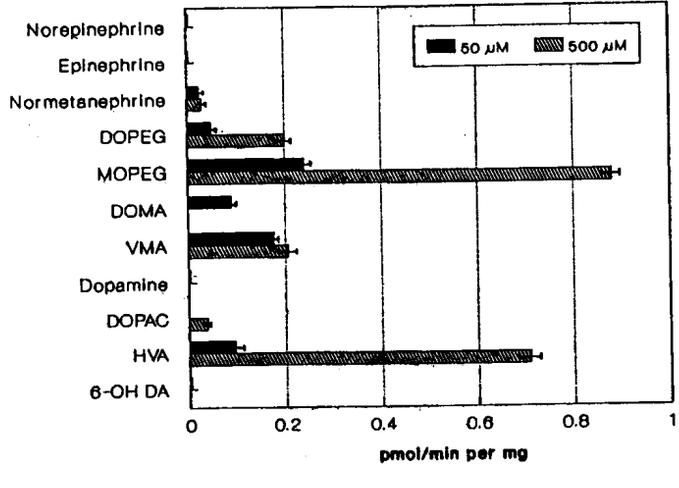

Fig. 1. Substrate specificity of phenol sulfotransferase from bovine brain cortex [52].
PST activity was assayed using 50 or 500 µM substrates: DOPEG, 3,4-dihydroxyphenylethylene glycol; MOPEG, 3-methoxy-4-hydroxyphenylethylene glycol; DOMA, 3,4-dihydroxymandelic acid; VMA, 3-methoxy-4-hydroxymandelic acid; DOPAC, 3,4-dihydroxy-phenylacetic acid; HVA, 3-methoxy-4-hydroxyphenylacetic acid; 6-OH DA, 6-hydroxy-

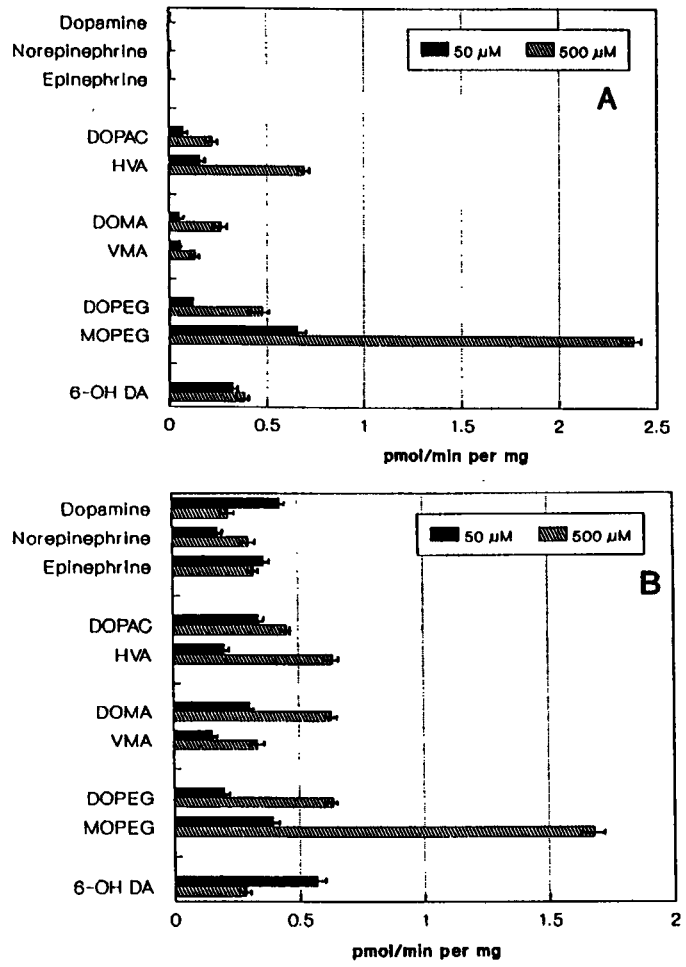

Fig. 2. Substrate specificity of phenol sulfotransferases from monkey brain cortex [50].
Cationic PST (A) and anionic PST (B) activity was assayed using 50 or 500 μM substrates. For abbreviations see Fig.1.

nervous system with essential water-soluble nutrients [58]. In the endothelial cells a number of metabolic pathways function and some drug-metabolizing enzymes are present [57, 59, 60], among them the enzymes that restrict the entry of circulating neurotransmitter and neuromodulator molecules into the central nervous system [61]. The barrier is thus recognized as a "dynamic regulatory interface" possessing both physical and enzymatic mechanisms for regulating passage of molecules from blood to brain [58].

As we have shown, brain microvessel endothelial cells also contain an aminopeptidase, acid hydrolases (β-galactosidase, acid phosphatase, sulfatase) and phenol sulfotransferase [62 - 64]. Our studies were performed on cultured microvessel endothelial cells which, as earlier demonstrated [65], can be used as a blood-brain barrier model system, *in vivo*.

We have found that both bovine and monkey (*Rh. macaca*) brain microvessel endothelial cells contain phenol sulfotransferase. The bovine enzyme, similarly as the bovine gray matter PST, is not active with catecholamines, but is active with their deaminated and 3-*O*-methylated metabolites (Fig. 4), [64, 66]. On the other hand, the monkey PST, like the anionic brain form, uses catecholamines as well as their metabolites (unpublished).

The blood-brain barrier location of PST suggests a role for the sulfation in regulation of catecholamine transfer between blood and the central nervous system. This is consistent also with the finding that most of the endogenous catecholamine neurotransmitters exist in human blood as sulfate esters, and this supports the implication that sulfate conjugation precedes the release of endogenous neurotransmitters into circulation [67].

The studied phenol sulfotransferases from brain endothelium cells are also active with exogenous phenols. Since sulfate-conjugation increases solubility in water of those toxic compounds, it also limits their movement across the blood-brain barrier and protects the brain. Thus, phenol sulfotransferase may represent a

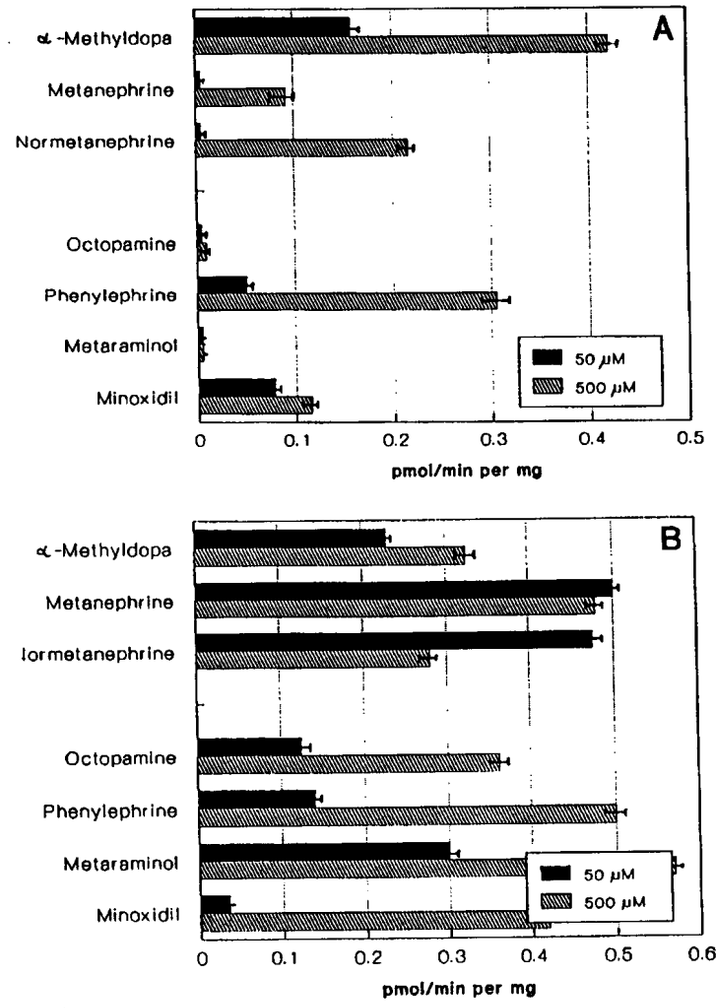

Fig. 3. Activity of monkey brain cortex PSTs with drugs [51]. Cationic PST (A) and anionic PST (B) activity was assayed using 50 or 500 μM substrates.

significant metabolic mechanism of the enzymatic blood-brain barrier.

Sulfation in male reproductive organs

As phenol sulfotransferase is one of the most important enzymes in detoxication and until now no attempt has been made to check the relationship between the properties of this enzyme and spermatozoa fertility, we have started studies on sulfation in the male reproductive system [68].

We have purified and characterized PSTs from bull and boar testis and isolated those enzymes from different parts of epididymides. There is only one PST form in bovine testis. It is thermostable and shows very high affinity towards *p*-nitrophenol, and much lower to-

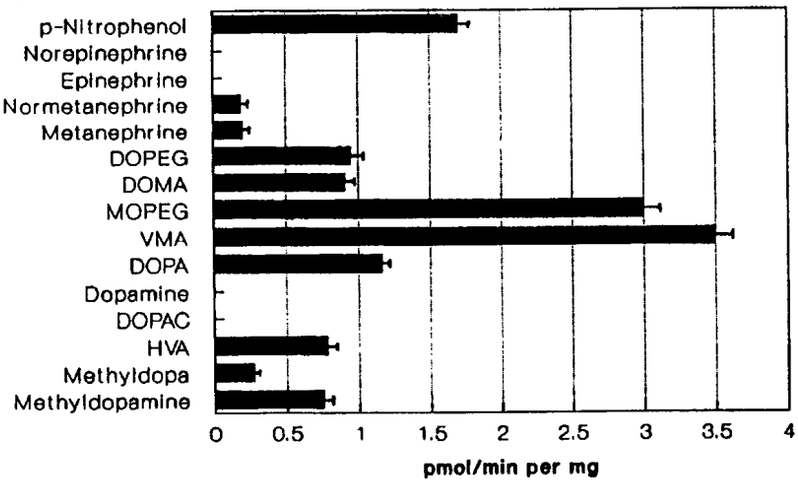

Fig. 4 . Substrate specificity of phenol sulfotransferase from bovine brain microvessel endothelial cell monolayers [66]. PST activity was assayed in 10-day-old monolayers using 500 μM substrates. For abbreviations see Fig. 1.

wards epinephrine. Unlike in the bull, the boar testis contains two different PSTs; one is thermostable and the other thermolabile. They both show higher affinity for *p*-nitrophenol than for epinephrine [68]. The three enzymes differ also in sensitivity to thiol group and arginine residue modifiers, as well as to 1-chloro-2,4-dinitrophenol, a specific inhibitor of sulfation. They seem to be different proteins responsible for inactivation of exo- and endogenous phenols. Whether species-dependent differences in sulfation are in any way correlated with spermatozoa fertility, remains still an open question.

In conclusion, phenol sulfotransferase is a part of the enzymatic mechanism protecting non-hepatic mammalian tissues against toxic exo- and endogenous substances. This enzyme (enzymes) is also involved in biotransformation of catecholamines, their derivatives and related drugs. Since sulfation can lead to inactivation or activation of some drugs, phenol sulfotransferase may influence to a significant extent their therapeutical activity. The differences observed in substrate specificity of PSTs from various species indicate that in omnivorous mammals both phenols and catecholamines, whereas in herbivorous mammals mainly exogenous phenols, are metabolized by this enzyme.

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