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Phenolsulphotransferase in human placenta

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Sulphate conjugation is an important pathway in the metabolism and excretion of catecholamines and many other phenolic compounds [1]. This reaction is catalysed by the cytoplasmic enzyme, phenolsulphotransferase (PST) which transfers the sulphate group from 3′-phosphoadenosine 5′-phosphosulphate (PAPS) to a phenolic acceptor [2]. PST is widely distributed in human tissues. It is highly active in the intestine, present in platelets and activity has also been noted in the placenta [3]. Placental PST may be important for the inactivation of endogenous phenols, dietary phenols or phenolic drugs present in the maternal circulation and prevent their access to the fetus.

PST in human platelets, brain and jejunum exist in two functional forms which we have denominated M and P forms [4]. PST M acts specifically on monoamines and related compounds, e.g. dopamine, tyramine, noradrenaline, adrenaline [5], α-methylnoradrenaline [6] and their metabolites such as 4-hydroxy-3-methoxyphenylglycol (HMPG) [4]; PST P acts on low concentrations of phenol [4]. Salicylamide, in low concentration, is also a specific P substrate although both it and phenol become M substrates at higher concentration [5, 7] (Bonham Carter *et al.*, submitted for publication). These two forms can be distinguished by their thermostability, the M form being more thermolabile, and by the action of the inhibitor, dichloronitrophenol (DCNP) which selectively inhibits the P form [4]. The two forms are controlled independently in different individuals [8]. Because of the possible functional importance of the placental enzyme, the aim of the present study was to study its activity towards a wide range of phenolic compounds and examine its sensitivity to DCNP, in order to determine whether both forms of the enzyme operate at this site and to measure their relative activities.

Materials and methods

A solution of 3′-phosphoadenosine 5′-phospho[^{35}S]sulphate (PAPS), 4.2 Ci/mmol, in 50% ethanol was purchased from New England Nuclear, Boston, Mass, USA and stored at −20°. Unlabelled PAPS was obtained from PL Biochemicals, Inc., Milwaukee, WI, USA. The substrates were purchased from Sigma Chemical Company, Poole, Dorset, U.K. and used without further purification. DCNP (2,6-dichloronitrophenol) was purchased from Fluka AG, Switzerland and also used without further purification.

Small segments from four freshly delivered human placentae were cut into smaller portions and as much blood as possible expelled from them. Homogenates (10% w/v) of each in 10 mM phosphate buffer, pH 7.4, were pooled and centrifuged at 100,000 *g* for 1 hr. The supernatant was stored in small aliquots at −20° in polypropylene test tubes.

The enzyme assay used was that described by Rein *et al.* [9] based on the method of Foldes and Meek [10]. The reaction mixture contained 100 μl 10 mM phosphate buffer, pH 7.4, 30 μM phenolic substrate (unless otherwise stated), 0.6 μM [^{35}S] PAPS and 10 μl placental supernatant. Inhibitor studies were carried out as described by Rein *et al.* [4]. A range of DCNP concentrations, from 10^{-4} to

Table 1. Substrate specificity and sensitivity of placental PST to DCNP inhibition

Substrate	Mean specific activity (pmoles/min/mg protein)	Relative activity	I_{50} with DCNP (M)
Dopamine	9.8	100	$>10^{-4}$
Tyramine	11.4	115	$>10^{-4}$
Noradrenaline	5.7	57	$>10^{-4}$
Adrenaline	5.9	59	$>10^{-4}$
HMPG	3.1	32	$>10^{-4}$
5-Hydroxytryptamine	3.1	31	$>10^{-4}$
Salbutamol	2.6	26	$>10^{-4}$
2,4-Dichloronitrophenol	2.6	26	10^{-5}
m-Phenylphenol	3.6	36	10^{-5}
Phenol	7.2	73	$<10^{-6}$
p-Cresol	5.8	59	$<10^{-6}$
o-Methoxyphenol	5.9	59	$<10^{-6}$
Resorcinol	6.2	62	$<10^{-6}$

10^{-8} M, was used with each substrate. All assays were carried out in duplicate and each value given was the mean of at least two determinations. Protein determinations were carried out by the method of Lowry *et al.* [11].

Results and discussion

Table 1 shows that placental PST is active with a wide range of phenolic compounds. Specific activity with tyramine and phenol was of a similar order to that previously observed with human platelets, about one tenth that of human jejunum, and at least ten times greater than that of human brain [4]. The relative order of activity values with various monoamine substrates was similar to that observed with the platelet using 30 μm concentrations [9]. Activity values with dopamine and tyramine were similar to each other, about double those with noradrenaline and adrenaline, whilst the enzyme was less active towards HMPG and 5-hydroxytryptamine. We have also used several synthetic phenols not examined previously and show that they, too, are actively sulphoconjugated by the placental enzyme.

The simplest way to distinguish between PST M and P substrates in human platelet and brain is using the selective P inhibitor, DCNP. At 10^{-5} and 10^{-6} M this compound almost totally inhibits phenol sulphoconjugation by these tissues, leaving activity towards tyramine and dopamine unimpaired [4]. This same pattern of selective inhibition was observed with the placental enzyme, phenol sulphoconjugation being substantially inhibited by 10^{-6} M DCNP, whereas the I_{50} with dopamine and tyramine was greater than 10^{-4} M. All other monoamine series substrates investigated, noradrenaline, adrenaline, HMPG, 5-hydroxytryptamine and salbutamol were similarly insensitive to DCNP, suggesting that they are also M substrates in the placenta. This same range of substrates has previously been shown to be M substrates in the platelet using another approach, of determining which activity values vary together in different individuals (Bonham Carter *et al.*, submitted for publication).

In previous studies, the only predominantly P substrates identified were phenol and salicylamide in low concentration. This present study has shown up several new PST substrates which, by their sensitivity to DCNP, also appear to be degraded by the P form of the enzyme. *p*-Cresol, *o*-methoxyphenol and resorcinol all behaved as phenol, whereas *m*-phenylphenol and 2,4-dinitrophenol showed intermediate sensitivity and were, presumably, mixed substrates.

In the platelet, 60 μM salicylamide and 600 μM paracetamol are both substrates for each form of the enzyme (Bonham Carter *et al.*, submitted for publication). Both were substrates for placental enzyme also and, here again,

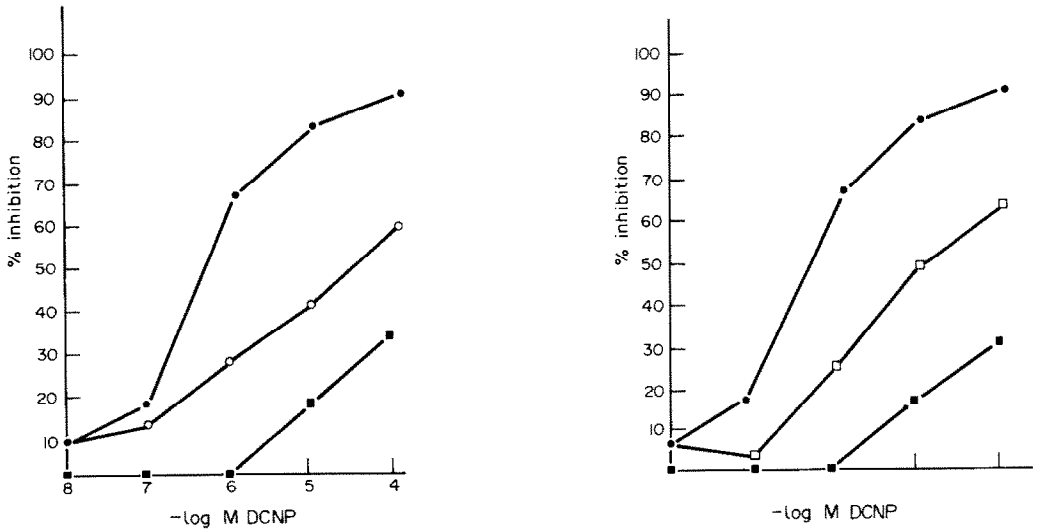

Fig. 1. Effect of DCNP on PST activity with paracetamol (600 μM) (○) and salicylamide (60 μM) (□) as substrates, compared with dopamine (■) and phenol (●).

DCNP sensitivity curves, which showed a pattern for each drug intermediate between that of phenol and dopamine (Fig. 1), show the enzyme to be similar to that of the platelet. Thus, all the evidence obtained so far supports the classification of placental PST into P and M forms, and suggests that they have similar properties to those elsewhere in the body. Whether maternal platelet activity reflects that in the placenta cannot be taken for granted, however, for a substantial proportion of a placental enzyme is presumably foetal in origin.

How may the findings reported here be of clinical relevance? Salbutamol is used to suppress premature labour and other β_2-adrenergic stimulating drugs used in this way, such as fenoterol and ritodrine, may also be substrates for PST. Paracetamol and salicylamide are also widely used in self-medication so that the activity of the placental enzyme will, to some extent, determine the extent to which they traverse the placental barrier. Thus, the two forms of the enzyme may help to regulate the amount of free drug gaining access to the fetus. This factor is of particular importance when questions of teratogenicity are raised, not only in its florid, anatomical forms, but also on a more subtle, behavioural level.

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The influence of a unilateral nephrectomy on the kallikrein activity of the remaining kidney and on the urinary kallikrein excretion in rats

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It has been suggested that the renal kallikrein-kinin system is responsible for the reduction of the tubular sodium reabsorption in a kidney after excision of the contralateral. This suggestion was based on the finding of increased kallikrein excretion after subtotal nephrectomy in rats [1]. It has been reported, however, that unilateral nephrectomized rats excrete less kallikrein than controls [2, 3]. To clarify this discrepancy, we investigated the influence of a unilateral nephrectomy on the urinary kallikrein excretion as well as on the kallikrein activity of the remaining kidney.

Methods. Male Wistar rats (200 g body weight) were anesthetized with ether. The left kidney was excised through a flank incision. Control rats were sham operated. Ten animals were alloted to each of five experimental (UNx) and five control (C) groups (100 animals). The rats were placed in collective cages and kept in a room at constant temperature and humidity (22°, 50%) with a 12 hr light–dark cycle. After a 3 days accommodation period to stainless steel metabolic cages, 24 hr urine was collected (in plastic beakers at room temperature) from a C and a UNx group 8, 15, 30, 60 and 90 days after surgery. At the end of the urine collection, the animals were anesthetized with pentobarbital (40–50 mg/kg body weight, i.p.) and a carotid artery was cannulated to measure blood pressure by means of a strain gauge. Then, the kidney(s) were excised. Kidneys and urine were kept frozen until assayed. Protein concentration in kidney homogenates was measured by the method of Lowry *et al.* [4], and creatinin in urine by the method of Popper *et al.* [5]. Urinary free aldosterone was estimated with a radioimmunoassay [6]. Plasma and urinary osmolarity was measured with a freezing point osmometer, and Na^+ and K^+ with an internal standard flame photometer. To determine the kallikrein activity, appropriately diluted urine and whole kidney homogenates were incubated with D-val–leu–arg–paranitroanilide (S2266, KabiDiagnostica, Stockholm, Sweden) for 120 min at 37° [7, 8]. A kallikrein unit (U) is the amount of enzyme capable of hydrolysing 1 μmole of substrate per