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Acidic catecholamine metabolites: substrates for human phenolsulphotransferase M

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Phenolsulphotransferase (PST; EC 2.8.2.1) catalyzes the transfer of a sulphate group from 3′-phosphoadenosine-5′-phosphosulphate (PAPS) to a phenolic acceptor substrate, forming the corresponding sulphate ester or conjugate. This is an important metabolic pathway for phenolic monoamines, some of their metabolites and for phenol and some dietary and pharmaceutical phenols [1].

Human platelet PST exists in two functional forms which we have termed PST P (specific for micromolar concentrations of phenol itself and other exogenous phenolic compounds) and PST M (specific for phenolic monoamines such as tyramine and dopamine) [2]. They also differ in tissue distribution, inhibitor sensitivity (the P form being selectively inhibited by 10^{-6} M 2,6-dichloro-4-nitrophenol (DCNP) [2]) and thermostability, the P form being more thermostable [2, 3]. The P form of the enzyme has been called by others TS (for thermostable), and the M form TL (for thermolabile) [3]. Activities of each are regulated independently [4] and have the same properties whether derived from human brain or platelet [5].

The radioenzymatic method that we have employed for assaying PST activity is based on that of Foldes and Meek [6] and uses radioactive ^{35}S-PAPS as sulphate donor, precipitating excess PAPS with barium hydroxide and zinc sulphate [4]. Recovery of the sulphate esters has been assessed for a range of phenolic substrates and is very good for many neutral and basic phenols [7]. In contrast, acidic substrates form insoluble barium salts which are precipitated with barium sulphate and excess PAPS and so cannot be assayed by this method [8, 9]. However, ion-exchange chromatography can be used to separate radioactive PAPS from the radiolabelled sulphated products of the acids [8–10] and has been used with rat PST [8, 9]. In this study we have used this method with 3,4-dihydroxyphenylacetic acid (DOPAC), 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid, HVA) and 4-hydroxy-3-methoxymandelic acid (vanilmandelic acid, VMA) to establish whether these compounds are substrates for either form of human platelet PST.

Materials and methods

Chemicals. Most reagents were obtained as previously described [4]. Ecteola cellulose (Cellex E) was purchased from Bio Rad Laboratories Limited, Watford, Hertfordshire, U.K.

Platelets. Platelets from normal volunteers were prepared from 10 ml of blood, as previously described [4], resuspended in 1 ml 10 mM phosphate buffer, pH 7.4, pooled, divided into aliquots and stored deep-frozen until use. Protein was measured using Lowry's method [11].

PST assay. PST activity towards tyramine and phenol was measured, using 10 μl of pooled platelet suspension in the radioenzymatic method previously described [4] based on that of Foldes and Meek [6], with ^{35}S-PAPS (final concentration 0.6 μM) as sulphate donor. Excess PAPS was removed by precipitation with barium hydroxide and zinc sulphate. All estimations were carried out in duplicate with a blank for each sample containing water instead of phenolic acceptor substrate. For the acid substrates, DOPAC, HVA and VMA, assay was carried out similarly to the end of the 10 min incubation period. The reaction was then terminated by transferring the contents of the tubes to 6 × 35 mm Ecteola cellulose ion exchange columns. Ion-exchange material had previously been equilibrated with 0.5 M ammonium bicarbonate and the columns washed with 3 ml 0.005 M ammonium bicarbonate before use. The radiolabelled sulphate esters of the acids were eluted with up to ten successive 1 ml fractions of 0.1 M ammonium bicarbonate which were collected into counting vial inserts containing 3 ml 'Instagel' and radioactivity counted in a Packard liquid scintillation counter. The products were eluted in fractions 2–5 for HVA, 1–4 for VMA and 2–7 for DOPAC. All estimations were carried out in duplicate with blanks for each sample containing water instead of phenolic acceptor substrate, and the appropriate blank fractions subtracted for each substrate.

K_m and V_{max} determinations. PST activity in pooled platelet samples was measured for the acidic substrates under investigation using various ranges from 3 μM to 3 mM final concentration. Higher concentrations caused an unacceptable lowering of pH of the incubation medium. Apparent K_m and V_{max} values were estimated using double reciprocal plots. PST activity in the same pooled platelet sample was measured with *p*-tyramine (17 μM) and phenol (7 μM) as substrates and V_{max} values calculated.

Inhibitor studies. DCNP was made up in the buffer used for assaying PST [4]. A range of DCNP solutions to give final concentrations of 10^{-4}–10^{-8} M was added to the incubation mixture immediately prior to assay. The acid substrates that were used appeared to be a substrate for PST, in that counts increased with the $BaSO_4$ method [4]. Appropriate substrate blanks were therefore subtracted.

Results and discussion

All three acids were good substrates for PST (Table 1), with considerably higher V_{max} values than those obtained with tyramine or phenol. They were all 0.75 mM. All double reciprocal plots gave straight lines. When experiments were performed at lower concentration ranges (10–100 μM), little or no activity could be detected. There

Table 1. V_{max} and K_m values of DOPAC, VMA and HVA with human platelet PST

Substrate	V_{max} (nmol/mg protein/10 min)	Relative specific activity	K_m (μM)
Tyramine	0.47	100	17*
Phenol	0.05	9.5	7.0†
DOPAC	1.22	260	3000
VMA	1.27	270	750
HVA	2.02	430	5000

* From Bonham Carter *et al.* [4]
† From Rein *et al.* [12].

was no evidence for a second enzyme metabolizing these substrates with a lower K_m. Figure 1 shows their sensitivity to inhibition by DCNP. All three acidic substrates showed a curve similar to that obtained with tyramine, which is quite distinct from that with phenol. Thus all three appear to be substrates largely or exclusively of PST M.

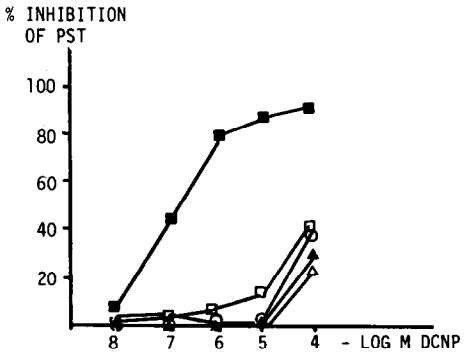

Fig. 1. Inhibition of human platelet PST by DCNP using tyramine □, phenol ■, HVA ○, VMA △ and DOPAC ▲ as substrates.

These results confirm those of Borchardt *et al.* [9] that the Ecteola cellulose method is satisfactory for the evaluation of HVA, VMA and DOPAC as substrates for PST. These acids are clearly good substrates for the human platelet enzyme, with higher V_{max} values than those for tyramine or phenol. In man, as we show here, they appear to be substrates for PST M, whereas in the rat, the findings of Baranczyk-Kuzma *et al.* [8] suggest that they are metabolized by a different form of the enzyme from that which acts on dopamine and noradrenaline. This finding points to a major difference in PST substrate specificity between man and rat. The recent results of Giorgi and Meek [13] also indicate that rat and human PST are substantially different.

Certain phenols are known to be substrates of both forms of human PST. Phenol itself, although specific for PST P at μM concentrations, becomes a substrate for PST M at higher concentrations with a K_m of about 0.5 mM [7]. Salicylamide is similarly a substrate for both forms of the enzyme being specific for the P form at μM concentrations and predominantly metabolized by the M form at mM concentrations [7]. HVA, VMA and DOPAC provided no evidence, however, for the existence of a second K_m at lower substrate concentrations, and the shape of the DCNP curves also pointed to metabolism by only one form. Thus there is no evidence for any significant sulphoconjugation of these acids by human PST P.

In man, all three acids studied here are partially excreted in conjugated form, although there are variations in degree. In one study, for example, DOPAC was found to be 81% conjugated, but HVA only 28% [14]; the extent to which such conjugation is with sulphate rather than glucuronic acid has not been firmly established. As shown here, both have a similar V_{max} and K_m with PST, so that differences of this order in degree or conjugation cannot be ascribed to differences in kinetic properties of the enzyme towards these two substrates. Differences in availability of enzyme and co-substrate at site of production of the acids seems a more likely explanation.

All three acids have a high K_m towards PST, in the mM range. This contrasts with low K_m values for adrenaline, noradrenaline and dopamine, all less than 10 μM [12]. These values may to some extent reflect the higher concentration of acids which the body is used to dealing with. The fact that the catecholamines, together with their acidic and other metabolites, are all substrates for PST M means that one can act as competitive inhibitor for the sulphoconjugation of another and, perhaps, play some role in the physiological regulation of enzyme activity.

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