Biochemical Pharmacology, Vol. 31, No. 10, pp. 1893–1897, 1982.
Printed in Great Britain.

0006-2952/82/101893-05 $03.00/0

MULTIPLE FORMS OF PHENOLSULPHOTRANSFERASE IN HUMAN TISSUES

SELECTIVE INHIBITION BY DICHLORONITROPHENOL

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(*Received* 7 *September* 1981; *accepted* 6 *January* 1982)

Abstract—Evidence is presented for two functional forms of phenolsulphotransferase in human tissues: (1) activity ratios, using dopamine and phenol as substrates, varied 30-fold between different tissues, whereas the dopamine to tyramine activity ratio was relatively constant; (2) incubation at 37° caused a selective decrease in activity towards dopamine compared with phenol; and (3) phenol sulphoconjugation was selectively inhibited by dichloronitrophenol and pentachlorophenol compared with that of dopamine and tyramine. The two forms, which have been designated M (monoamines) and P (phenol), were both present in platelets, jejunum, adrenal and brain.

Phenolsulphotransferase (PST) catalyses the transfer of sulphate from 3′-phosphoadenosine 5′-phosphosulphate (PAPS) to a wide array of acceptors, ranging from phenolic drugs to endogenously synthesized biogenic amines and their metabolites [1]. Recent publications have raised the possibility of the products of this reaction having physiological functions as precursors for further reactions [2] and transport forms of the parent phenol [3], apart from their more traditional role as excretory end-products [4, 5]. Interest in human PST has recently sharpened [6]: the detection of significant activity in brain [7, 8], blood platelets [9–11] and other peripheral sites [12, 13] has opened up the possibility of obtaining from the peripheral blood clinically useful information [14, 15] about an enzyme likely to be important for the normal functioning of monoamine systems. The underlying assumption behind such clinical investigations is that the properties of the platelet enzyme resemble those occurring elsewhere in the body. However, only one study [13] has so far directly compared PST in different human tissues: using 4-hydroxy-3-methoxyphenylglycol (HMPG) as substrate, the authors showed that platelet, gut and kidney enzymes have similar pH optima and K_m values. A parallel between these peripheral enzyme sources and the brain has not yet been established.

Most previous work with PST has been on rat tissues and several protein forms [16–20] have been purified with different substrate specificity. Here we present the first evidence for two functionally different forms of PST in man, which we have termed M (monoamines) and P (phenol) activities. Both were present in all tissues examined.

MATERIALS AND METHODS

Human tissues. Platelets were harvested from four healthy volunteers (2 male and 2 female), taking no drugs, prepared as previously described, pooled and stored frozen at −20° [11]. Normal adrenal tissues were obtained at operation from two patients undergoing removal of phaeochromocytoma and from one patient with a parenchymal tumour. Normal jejunum was obtained by biopsy from four patients being screened for coeliac disease but found to be normal. Homogenates (10%, w/v) of jejunal and adrenal tissues were prepared in 10 mM phosphate buffer (pH 7.2) using an all metal Ultra-Turrax homogenizer and stored frozen at −20°. Homogenates from the same tissue were pooled prior to assay and diluted (1/10 for adrenal and 1/30 for jejunum) in 10 mM phosphate buffer, pH 7.2.

Four brains were obtained post-mortem, and were kindly supplied by Dr. Grey of High Wycombe General Hospital, U.K. The delay before autopsy and storage at −20° was less than 24 hr in all cases. The patients had all died from heart disease and had no known neurological disorder. Homogenates (20%, w/v) of frontal cortex in 10 mM phosphate buffer, pH 7.2, were prepared and centrifuged at 30,000 *g* for 30 min and stored at −20°. They were pooled prior to assay and volumes containing approximately 200 μg protein were used in all subsequent experiments.

Phenolsulphotransferase assay. PST was assayed using the method of Foldes and Meek [21] as modified by Rein *et al.* [11]. All assays were performed at pH 7.2 using ^{35}S-PAPS in a final concentration of 0.4 μM. Of each sulphate acceptor 30 μM was used unless stated otherwise. For assays using homogenates but not supernatants, 1 mM pargyline was included to inhibit monoamine oxidase. For each, sample blanks were included with ^{35}S-PAPS but with buffer instead of the sulphate acceptor. All assays

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were carried out in duplicate. For all the work described, experiments were carried out two to three times, the results given being the mean values obtained.

Thermostability. Pooled platelet samples and frontal cortex supernatants were preincubated in an incubation mixture with buffer and sulphate acceptor for varying lengths of time at 37° before the reaction was started with ^{35}S-PAPS. Inhibition was expressed as a percentage of activity obtained using samples without preincubation.

Inhibitor studies. For these studies, pooled 30,000 *g* supernatants from each tissue were used and stored at −20°. Substrate concentrations of twice the apparent K_m values previously reported in the various tissues were used [22]. For platelets, final concentrations of dopamine, phenol and tyramine were 6, 14 and 180 μM, respectively. Brain tissue was assayed using 6 μM dopamine and 20 μM phenol whereas jejunum was assayed with 4 μM and 10 μM of these substrates, respectively. Dichloronitrophenol (DCNP) and pentachlorophenol (PCP) were present during a 20 min preincubation at 37° and during assay incubations. For each tissue, inhibitor blanks were obtained using different concentrations of DCNP or PCP in the absence of phenolic substrate.

Protein determinations. Protein concentrations were estimated using the method of Lowry *et al.* [23] with bovine serum albumin as standard.

Materials. ^{35}S-PAPS (0.4–4.4 mCi/μmole) was obtained from New England Nuclear Corp. (Boston, MA) and 100 μl aliquots of this were stored at −20°. All sulphate acceptor substrates were obtained from Sigma Chemical Co. (London, U.K.). DCNP and PCP were purchased from Koch–Light Laboratories Ltd. (Colnbrook, U.K.) and Aldrich Chemical Co. Ltd. (Gillingham, U.K.), respectively. Instagel was purchased from Packard Instrument Co. (Wembley, U.K.).

RESULTS

The assay was found to be linear with tissue concentrations over a limited range of protein which varied with the particular tissue concerned (Fig. 1). At higher concentrations, no further increase of activity was obtained by increasing the amount of tissue. Appropriate protein concentrations for each tissue were therefore used in all subsequent experiments. The enzyme assay was found to be linear with time up to 12 min of incubation, using dopamine and phenol as substrates.

Table 1 shows the specific activity of PST in different human tissues employing dopamine, tyramine and phenol as substrates (all at 30 μM). Jejunum was by far the most active toward tyramine and dopamine, followed by platelets, adrenal and frontal cortex in descending order. With phenol, the adrenal was more active than the platelet. The specific activity in the frontal cortex was very much lower than in the other tissues and was measured in a high speed supernatant rather than homogenate, for this reason. The specific activities given for this are therefore not strictly comparable with the other three. Nonetheless, useful information can still be obtained from the substrate activity ratio. The ratio data in Table 1 indicate that although dopamine/tyramine ratios remained relatively constant from tissue to tissue, dopamine/phenol ratios differ substantially, ranging from 0.18 in the adrenal to 5.6 in the jejunum.

In an attempt further to distinguish between dopamine and phenol-conjugating activity, enzyme thermostability was assessed in platelets and brain. The results are shown in Fig. 2. In both tissues activity towards dopamine was much more heat-sensitive than that towards phenol.

Further attempts were made to distinguish dopamine and phenol conjugating activity with the use of selective inhibitors. In these studies supernatants

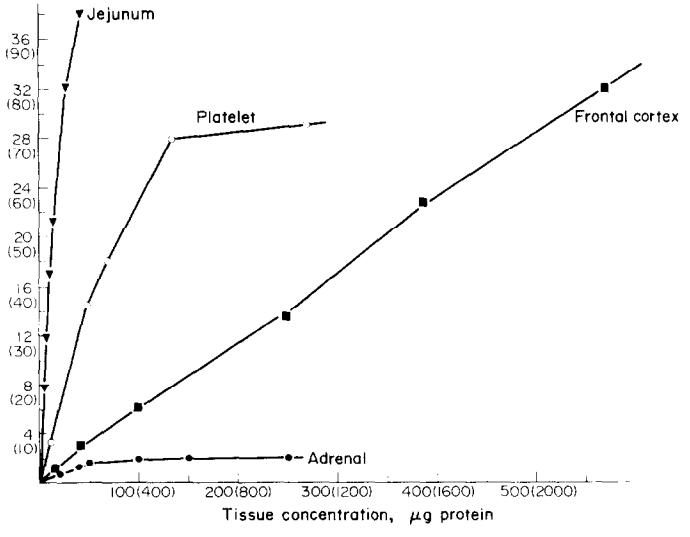

Fig. 1. Effect of tissue concentration on phenolsulphotransferase activity, assayed with 30 μM dopamine. Tissues were prepared as described in Materials and Methods. The ordinate represents the amount of sulphate conjugate formed, expressed as cpm × 10^3. The numbers in parentheses along both axes apply to the frontal cortex.

Table 1. Phenolsulphotransferase activity and activity ratios in human tissues

Tissue	Specific activity			Ratios	
	Dopamine	Tyramine	Phenol	Dopamine/tyramine	Dopamine/phenol
Jejunum	138	105	24.5	1.3	5.6
Platelet	22.1	20.7	8.9	1.1	2.5
Adrenal	1.9	1.9	10.4	1.0	0.18
Frontal cortex	0.66	0.26	0.68	2.5	1.0

Details of tissue preparation and enzyme assay conditions are described in Materials and Methods.

rather than homogenates were used in order to eliminate any possible interference by mitochondrial monoamine oxidase. The sulfhydryl reagents, *p*-chloromercuribenzoate, *N*-ethylmaleimide and iodoacetate, at 1 μM concentrations, brought about no change in dopamine/phenol ratio of human platelets, whereas higher concentrations resulted in total inhibition of activity toward both substrates. DCNP and PCP, however, effected a marked increase in this ratio. The data in Fig. 3 indicate that in human platelets, these reagents selectively inhibited phenol sulphoconjugation, leaving dopamine and tyramine conjugation relatively unimpaired. Thus, at 10^{-6} M DCNP and PCP concentrations, dopamine conjugation was inhibited by 5% whereas activity toward phenol was inhibited by 100%. Similar curves were obtained with DCNP in frontal cortex and jejunum, as shown in Fig. 4.

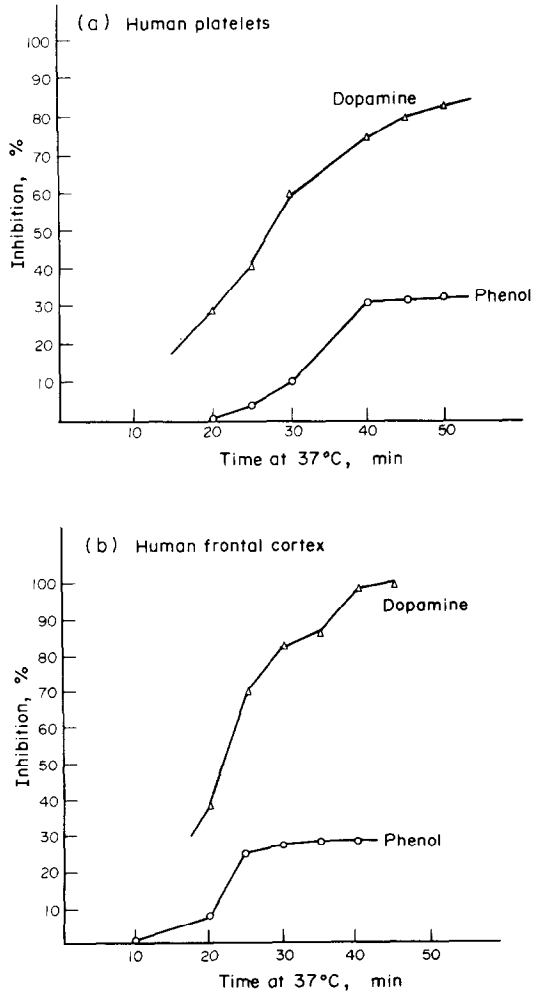

Fig. 2. Thermostability of phenolsulphotransferase from (a) platelets and (b) frontal cortex. The enzyme activity data are expressed relative to values obtained without preincubation at 37°.

DISCUSSION

The preferential inhibition of phenol compared with monoamine sulphation by DCNP, together with

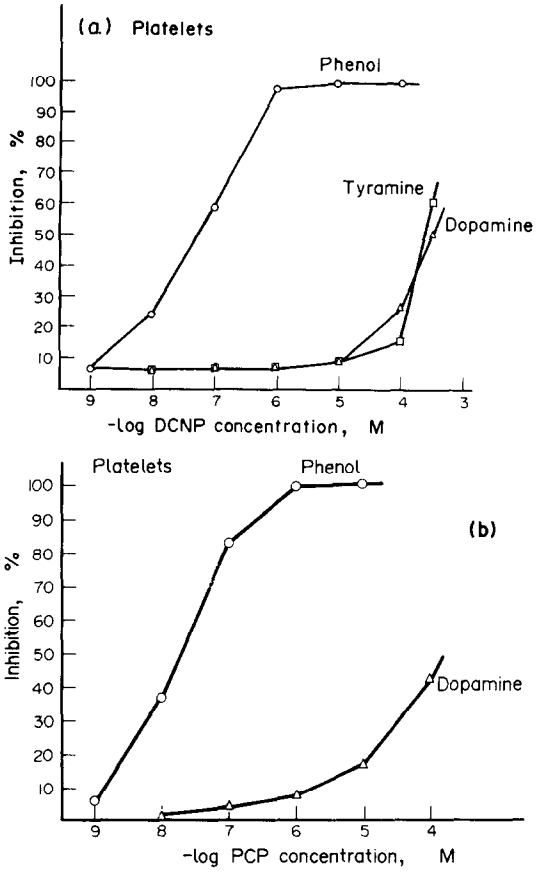

Fig. 3. Inhibition of platelet phenolsulphotransferase by various concentrations of (a) dichloronitrophenol (DCNP) and (b) pentachlorophenol (PCP). Results are expressed relative to values obtained in the absence of inhibitors.

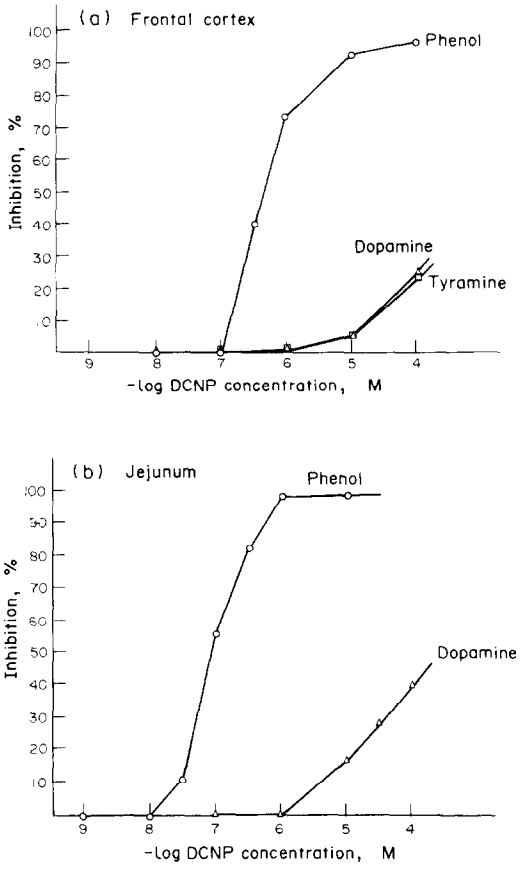

Fig. 4. Inhibition of phenolsulphotransferase from (a) frontal cortex and (b) jejunum, by various concentrations of dichloronitrophenol (DCNP). Results are expressed relative to values obtained in the absence of inhibitors.

the differential tissue distribution and thermostability findings, provide strong evidence that there are two functional forms of human PST, one specific for phenol (P enzyme) and one specific for dopamine and tyramine sulphoconjugation (M enzyme). The thermostability curves in Fig. 2 suggest the possibility that the enzyme that is active towards dopamine might account for 20–30% of the activity towards phenol rather than there being two separate absolutely specific enzymes. However, the inhibitor curves in Fig. 3 and Fig. 4 do not support this interpretation as in both jejunum and platelets, 10^{-6} M DCNP inhibited phenol-conjugating activity by 100% while inhibiting activity towards dopamine by less than 10%. This degree of differential inhibition suggests that the two substrates are remarkably specific for determining P and M activities. Further work will clearly be needed to characterize the substrate specificities of each form in greater detail and to determine whether the two forms correspond to two distinct proteins.

Although this is the first report of two functional forms of PST in man, there is evidence for heterogeneity of the enzyme in the rat. Jones and his coworkers [16, 17] separated two protein fractions from rat liver, one of which was relatively more active towards *p*-nitrophenol and the other towards L-tyrosine methyl ester. More recently Jakoby's group [18] have extensively purified four different proteins from rat liver, all of which sulphoconjugate 2-naphthol. Although at least two of these appear rather similar to each other and have similar substrate specificities, one enzyme form does differ in both protein and kinetic properties from the others. Branczyk-Kuzma *et al.* [20] have also found that the substrate specificity pattern for PST obtained with rat brain homogenates differs markedly from that with rat liver, the former being relatively more active towards the monoamine metabolites than the parent monoamines. More work will be needed to determine whether the two functional forms of PST in man described here correspond to two distinct forms in the rat.

The rat may not be a good model for studying sulphoconjugation in man. In this study, we have found that the thiol blockers, which distinguished two forms of the rat enzyme, were able to inhibit human PST but did not act selectively on phenol or dopamine-conjugating activity. Other differences in physicochemical properties of the enzyme have been noted between the two species. The pH optimum for catecholamines in rat tissues is 9.0 [21, 26], whereas in human tissues we have noticed a decrease in enzyme activity toward these substrates at values greater than physiological pH (unpublished observations). We have previously reported that human PST is substantially more active toward monoamines than their acidic metabolites [11, 22] whereas the reverse is true in the rat [21, 26]. In addition, the excretion patterns of conjugated amines and their metabolites differ markedly in the two species [27].

The relatively low activity of PST observed here in human brain, may reflect its relatively minor role in the inactivation of biogenic amines in this tissue, although post-mortem deterioration may well contribute, to an unknown extent, to these low values. We have previously calculated that despite the particularly low K_m value of PST for the catecholamines, oxidative deamination by monoamine oxidase is likely to be more important in metabolizing physiological concentrations of these substrates in the brain [22]. Renskers *et al.* [7] have drawn the same conclusion, based on low PST activities in human brain which they also determined.

The specific activities of PST toward dopamine, tyramine and phenol may be affected by the varying solubilities of their respective sulphate conjugates in barium hydroxide, since an 18% variation in recoveries has been previously reported using these substrates [21]. Nonetheless, these recoveries should be constant from tissue to tissue and should, therefore, not affect the ratio data. It is still possible, however, that the different tissues contain varying amounts of sulphatases with differential activity toward dopamine and phenol. Although this might affect the ratio data, it would not be expected to affect the sensitivity of the two forms toward DCNP. It also seems unlikely that three independent criteria for distinguishing the two forms of PST would be affected in parallel by these factors.

The data reported here demonstrate that both the P and M forms of human PST are present in each of the three tissues examined, although in different

proportions. As $K_{i\,50}$ values for phenol and dopamine are quite similar in the different tissues, these findings, together with previously reported similarities between human platelet, jejunum and kidney PST [10, 13], suggest that the platelet enzyme may well mirror M and P enzymes elsewhere in the body. It follows that clinical studies on PST M and P activities in this tissue can now be carried out to investigate individual variations in phenolic drug metabolism and may, in addition, help to shed light on disturbances of monoamine metabolism in psychiatric disorders.

Acknowledgements—G. R. was supported by a grant from the Cancer Research Campaign.

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