Species and Organ Differences of Sulphate Conjugation of \( p \)-Nitrophenol in Liver and Platelets

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Sulphate conjugation of \( p \)-nitrophenol (\( p \)-NP) in the liver and platelet cytosol of guinea pigs, rabbits and dogs were studied. The dependency of phenol sulphotransferase (PST) activity on \( p \)-NP concentration in the liver of guinea pigs and rabbits and in the platelets of guinea pigs were similar to that reported for the liver (Mizuma et al., J. Pharmacol. 

Keywords: phenol sulphotransferase; species difference; liver; platelet; \( p \)-nitrophenol

Introduction

Sulphate conjugation is a major metabolic pathway for the disposition of phenolic drugs in the bodies of animals as well as of humans. Correlations have been reported between phenol sulphotransferase (EC 2.8.2.1) (PST) activities in human platelets and psychiatric disease,\(^1\), and between thermostable PST activity in human platelets and urinary excretion of acetaminophen sulphate after oral dosing.\(^2\). However, the real PST activity was not assessed in these studies because of a variation in absorption of orally dosed acetaminophen.

A good correlation between PST activity on \( p \)-nitrophenol (\( p \)-NP) in the \( \mu \)M order in the liver and platelets of rats was reported by our group.\(^3\) Over the wide range of \( p \)-NP concentration from 0.1 \( \mu \)M to 10 mM, the PST activity of platelet cytosol versus \( p \)-NP concentration was biphasic and similar to that of the liver cytosol in rats; that is, PST showed activities on \( p \)-NP in both the \( \mu \)M and mM orders.\(^4-5\) Only one type of PSTs purified from rat liver showed activity on \( p \)-NP in the \( \mu \)M order and on tyramine.\(^6\) Moreover, sulphate conjugation of acetaminophen in the rat liver was found to be catalyzed mainly by the PST active on \( p \)-NP in the \( \mu \)M order.\(^7\)

The liver is a primary organ for sulphate conjugative metabolism in the body, and the platelets can be easily obtained without surgical manipulation for assaysing PST activity. Estimation of the PST activity in the body by assaying the PST activity of tissues, which can be easily sampled, would be clinically useful. If the PST activity in animals correlates with that in humans, its measurement in animals would be helpful in drug development experiments. In the present report, the PST activity on \( p \)-NP in animals was studied to determine the relationship in PST activity between liver and platelets and among animals.

Materials and Methods

\( p \)-NP and \( 3^{35} \)S phosphoethanolamine \( 5^{35} \)S phosphosulfate (\( [35S] \)PAPS), 1.0–1.5 Ci mmol\(^{-1}\) were obtained from Wako Pure Chemicals, Tokyo, Japan and New England Nuclear, Boston, MA, U.S.A. Bovine serum albumin (BSA, fraction V) and dithiothreitol were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. All other chemicals and reagents were of analytical grade or better.

Liver cytosol and platelet cytosol were prepared according to the method of Nakamura et al.\(^8\) Hartley male guinea pigs (5–6 weeks, ca. 330g), New Zealand white male rabbits (12–14 weeks, ca. 2.5 kg) and beagle male dogs (12–20 weeks, ca. 9 kg) were used. In this study, beagle dogs were supplied only for the experiment of platelet PST. The PST activity of liver and platelet cytosol was measured by the method of Anderson & Weinschilboum\(^9\) as described by Nakamura et al.\(^9\)

Results

The profiles of the PST activity versus \( p \)-NP concentration are shown in Figure 1. The activity increased in a biphasic manner with increasing \( p \)-NP concentration. The biphasic nature of the activity is similar to that observed in human platelets and liver cytosol. The PST activity was also measured in the liver and platelets of rats, guinea pigs, rabbits, and dogs. The results indicated that the PST activity was higher in the liver than in the platelets in all species tested.

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titation in Figs. 1a, b and c showed that p-NP sulphate conjugation in liver cytosol of guinea pigs and rabbits were similar to that in the liver cytosol of rats. That is, there was one peak of PST activity in the μM order of p-NP (around 1 to 10 μM). The PST also showed activity on p-NP in the mM order.

The PST activity versus p-NP concentration profile for guinea pig platelets was similar to that for rat platelets (Figs. 1d and e). There was one peak of the PST activity on p-NP at the concentration of 1 to 10 μM, and the PST activity increased again with an increase in p-NP concentration above 1 mM. On the other hand, the PST activity versus p-NP concentration profiles for the platelets of rabbits and dogs were different from that for rat platelets (Figs. 1d, f and g). The PST activity in the platelets of rabbits and dogs on p-NP at the concentration of 1 to 10 μM was much lower compared with that at the mM order.

Discussion

The correlation in PST activity between liver and platelets in each species was considered as follows. The profile of the PST activity on p-NP in the liver and platelets of rats was biphasic and similar, where sulphate conjugation with substrate inhibition in the μM order was catalyzed by the PST IV5 described in the Introduction. The rat brain PST activity on p-NP in the μM order with substrate inhibition was also observed by Baranycz-Kuzuma et al.9 These observations indicated a similarity in PST activity on p-NP in all these organs in rats.

In guinea pigs, the profile of the PST activity on p-NP of liver and platelets was also biphasic and similar, suggesting the existence of PST, at least, similar to rat PST IV. In rabbits, however, the profile of the PST activity in the liver and platelets was not similar, indicating an organ difference of the PST on p-NP.

In the platelets of dogs, the PST activity on p-NP at the mM order was much higher compared with that of rats, guinea pigs and rabbits, suggesting the contribution of the PST in the platelets to an extrahepatic metabolism in the dogs.

This comparative study on the substrate concentration specificity of PST on p-NP, especially in the μM order of p-NP, showed species and organ differences in PST activity. The biphasic activities of PST on p-NP in the platelets and liver of rat and guinea pig were similar to the activities in humans reported by other researchers.10 Further study of the PST in rats and guinea pigs on the other substrates is required for the selection of model animals for sulphate conjugation metabolism in humans.

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References