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RAT PHENOL SULFOTRANSFERASE

ASSAY PROCEDURE, DEVELOPMENTAL CHANGES, AND GLUCOCORTICOID REGULATION*

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Abstract—Phenol sulfotransferase (PST) catalyzes the sulfate conjugation of phenolic monoamines and phenolic drugs. It has been difficult to measure PST activity in tissue homogenates accurately because of the presence of potent endogenous PST inhibitors. Optimal conditions were determined for the assay of rat PST in very dilute tissue homogenates. These conditions negated the effects of endogenous enzyme inhibitors. Apparent K_m values for 3-methoxy-4-hydroxyphenylglycol, the sulfate acceptor substrate used, were 0.15, 0.14, and 0.02 mM for liver, kidney, and brain homogenates respectively. Apparent K_m values in the same tissues for 3'-phosphoadenosine-5'-phosphosulfate, the sulfate donor, were 0.11, 0.07, and 0.07 μM respectively. Rat PST activity expressed per mg protein increased 6.3-fold in the liver, 6.6-fold in the brain, and did not change in the kidney between birth and 10 weeks of age. There was a 5-fold increase in kidney PST activity in both adrenalectomized and sham-operated Sprague–Dawley rats after treatment with dexamethasone (7 μmoles/kg daily for 3 days). Brain enzyme activity was unchanged and liver PST activity increased only 41% during 72 hr of daily treatment with dexamethasone. Basal enzyme activities in all three tissues were no different in adrenalectomized and sham-operated animals. The increase in rat kidney PST activity in response to dexamethasone was dose dependent, and treatment of animals with cycloheximide, a protein synthesis inhibitor, blocked the elevation of kidney PST activity after dexamethasone. Treatment of eight inbred and two outbred rat strains with dexamethasone resulted in striking increases in renal PST, smaller increases in liver PST, and no changes in brain enzyme activity in all ten strains.

Phenol sulfotransferase (EC 2.8.2.1, PST) catalyzes the sulfate conjugation of many monoamines, monoamine metabolites and phenolic drugs [1–3]. Although PST plays an important role in the metabolism of a variety of endogenous compounds and drugs, the regulation of the enzyme activity is not well understood. This is true in part because past attempts to assay PST activity in tissue homogenates have been hampered by the presence of potent endogenous PST inhibitors [4]. Interest in the regulation of PST has increased recently because of the discovery that the enzyme activity is present in homogenates of an easily obtained human tissue, the blood platelet [5–8]. There are large individual differences in human platelet PST activity, differences that correlate significantly with individual variations in the enzyme activity in other tissues such as the kidney [7, 8]. These observations have raised the possibility that variations in PST activity might represent one factor in functionally significant differences in sulfate conjugation *in vivo*. This possibility has increased the importance of understanding the regulation of PST activity in both humans and experimental animals. The following experiments were undertaken to study the possible roles of age, humoral factors, and inheritance on the regulation of PST activity in the rat. These studies were made possible by the development of an assay procedure that negated the effects of tissue PST inhibitors. The results of these experiments will serve as a foundation for future studies of the regulation of PST and of the variations in the enzyme activity as one factor influencing variations in the sulfate conjugation of monoamine neurotransmitters and of phenolic drugs.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (normal, adrenalectomized, and sham-adrenalectomized) and pregnant female Sprague–Dawley rats were obtained from the Hormone Assay Corp., Chicago, IL. Lewis, MAXX, Fischer-344, Buffalo, and ACI male rats were purchased from Microbiological Associates, Inc., Rockville, MD. Spontaneously hypertensive and Wistar–Kyoto male rats were obtained from the Laboratory Supply Co., Indianpolis, IN. Male Wistar–Furth rats were purchased from ARS Sprague–Dawley, Inc., Madison, WI, and male Long–Evans rats were obtained from the Charles River Breeding Lab-

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oratories, Inc., Wilmington, MA. Normal animals were fed standard laboratory chow and given free access to water. Adrenalectomized animals were also given free access to 0.9% saline. In all experiments except those involving studies of growth and development, animals were 10–12 weeks of age at the time they were killed.

Tissue preparation

Rats were decapitated and exsanguinated. The liver, kidneys, and brain were removed and frozen on aluminum foil on dry ice. The tissues were then placed in 9 vol. of ice-cold 5 mM potassium phosphate buffer, pH 7.5, and were homogenized for 15 sec with a Polytron homogenizer. The homogenates were centrifuged at 10,000 *g* for 10 min, and the supernatant fractions were centrifuged at 100,000 *g* for 60 min. All centrifugation steps were performed at 4°. The supernatant fractions after centrifugation at 100,000 *g* for 60 min were diluted with 5 mM potassium phosphate buffer, pH 7.5, that contained 0.125% bovine serum albumin (BSA). The dilutions were (v:v): liver, 1:8,000; brain, 1:160; and kidney, 1:160. Therefore, the final dilutions were 1:80,000 for liver and 1:1,600 for brain and kidney. This high degree of tissue dilution was required to overcome the effects of tissue inhibitors of PST [4].

PST assay

PST activity was measured by a modification of the method of Foldes and Meek [9]. The sulfate acceptor substrate was 3-methoxy-4-hydroxyphenylglycol (MHPG), and the sulfate donor was [^{35}S]-3′-phosphoadenosine-5′-phosphosulfate (PAPS). The assay was performed with 200 μl aliquots of diluted supernatant fluids. Blank samples contained no MHPG and active samples contained substrate. In the case of liver and kidney homogenates, 10 μl of 7.5 mM bis-MHPG piperazine was added to each active sample. Ten microliters of 1.9 mM bis-MHPG piperazine was added to each active sample for brain homogenates. The use of these substrate concentrations resulted in final MHPG concentrations of 0.5 mM for liver and kidney and 0.125 mM for brain. The enzyme reaction was initiated by the addition of 100 μl of mixture of the following reagents (final concentration in 310 μl indicated): potassium phosphate buffer, 8 mM; dithiothreitol, 8 mM; and [^{35}S]PAPS, 0.4 μM. The pH of the phosphate buffer was 6.5 for liver, 6.0 for brain, and 7.0 for kidney homogenates. The reaction mixture was incubated at 37° for 30 min in a shaker water bath, and the reaction was terminated by the addition of 200 μl of an equal volume mixture of 0.1 M barium hydroxide and 0.1 M barium acetate followed immediately by the addition of 100 μl of 0.1 M zinc sulfate. The reaction tubes were vortexed and were centrifuged at 800 *g* for 10 min. An additional 100 μl of 0.1 M barium hydroxide and 100 μl of 0.1 M zinc sulfate were added to the tubes in succession, and the vortex and centrifugation steps were repeated. Five hundred microliters of the final supernatant was removed and was placed in a 6 ml polyethylene scintillation counting vial that contained 5 ml Instagel (Packard Instrument Co., Inc. Downers Grove, IL). Radioactivity was measured in a Packard 3385 liquid scintillation counter. One unit of enzyme activity represented the formation of 1 nmole of MHPG sulfate per hr. The results were expressed either per g tissue or per mg protein.

Assays of partially purified rat liver and rat brain PST were performed in exactly the same fashion except that 10 μl of the partially purified enzyme in 200 μl of 0.125% BSA in 5 mM potassium phosphate buffer, pH 7.5, replaced the diluted high-speed supernatant fractions of tissue homogenates. All assays for studies of the effects of growth and development on PST activity and the effects of hormones on the enzyme activity were performed with fresh tissue homogenates. Undiluted high-speed supernatant fractions of tissue homogenates were stored at −85° for some of the initial experiments in which the assay conditions were established. No frozen preparation was ever reused after it had once been thawed.

Protein assay

Protein concentrations were measured by the dye-binding method [10] with BSA as a standard.

Thin-layer chromatography

The products of the reaction were identified by thin-layer chromatography on Eastman Chromagram sheets of silica gel with a fluorescent indicator. The procedure used has been described in detail elsewhere [4].

Purification of rat liver and brain PST

PST activity was partially purified from homogenates of Sprague–Dawley rat liver and brain by a modification of the method of Anderson and Weinshilboum [4]. Tissues were prepared as described above and solid ammonium sulfate was added to high-speed supernatant fractions to a final saturation of 30%. After centrifugation of the resulting suspension at 16,300 *g* for 10 min, the precipitate was discarded and solid ammonium sulfate was added to the supernatant fraction to a final saturation of 50%. The centrifugation step was repeated and the resulting precipitate was dissolved in a minimal volume of 5 mM potassium phosphate buffer, pH 7.5. The dissolved precipitate was applied to a Sephadex G-100 column equilibrated with 50 mM potassium phosphate buffer, pH 7.5, and the fractions with the highest specific activities of PST were pooled and concentrated in an Amicon pressure concentrator with a PM10 membrane. The specific activities of these partially purified preparations were 91.2 units/mg protein for liver and 8 units/mg protein for brain. These specific activities represented 3- and 3.7-fold purifications over those present in 100,000 *g* supernatant fractions for liver and brain respectively.

Although much greater degrees of purification of PST after the use of similar procedures have been reported [4], the initial assays of enzyme activity were performed with concentrated tissue homogenates, conditions that result in striking inhibition of PST by endogenous enzyme inhibitors. Therefore, a large part of the apparent purification of the enzyme probably resulted from removal of inhibitors during the purification procedure. Since the enzyme activity in our tissue homogenates was assayed under

conditions of high dilution that did not result in inhibition of PST, calculations of the degree of purification were not elevated artifactually due to removal of inhibitors.

Kinetic analysis

Michaelis–Menten (K_m) values were determined by the method of Wilkinson [11] with a Fortran program written by Cleland [12] and by the method of Eisenthal and Cornish-Bowden [13]. A Control Data Corporation 3500 computer was used for these calculations.

Materials

[^{35}S]PAPS (0.4 to 3.3 Ci/mmole) was purchased from the New England Nuclear Corp., Boston, MA, and was stored at −85° in 200 μl aliquots. Bis(3-methoxy-4-hydroxyphenylglycol) piperazine salt, dithiothreitol (Cleland's reagent), cycloheximide and bovine serum albumin were purchased from the Sigma Chemical Co., St. Louis, MO. MHPG sulfate was obtained from the Tridom Co., Hauptague, NY. Dexamethasone was obtained from Elkins-Sinn, Inc., Cherry Hill, NJ. Dye reagent for protein assay was purchased from Bio-Rad Laboratories, Richmond, CA.

RESULTS

Assay conditions

Introduction. A serious limitation of previously described assay procedures for tissue homogenate PST was failure to take into account the effects of potent endogenous PST inhibitors [4]. One goal of this series of experiments was to develop an accurate assay for PST activity in rat tissue homogenates that negated the effects of these inhibitors. This goal was accomplished by taking advantage of the high specific activity of [^{35}S]PAPS to make it possible to use very dilute homogenates, homogenates so dilute that inhibitors had no significant effect under the conditions of the assay.

Effect of increasing tissue concentration and incubation time. PST activity increased in a linear fashion with increasing quantity of tissue homogenate for all three tissues studied (Fig. 1). The relationship between PST activity and tissue concentration remained linear up to at least 8, 400, and 400 μg of tissue per assay for liver, brain, and kidney respectively. In all cases, the quantity of tissue used in the assay was well within the linear range. The linear relationship between enzyme activity and tissue concentration was not trivial. Lack of such a relationship initially drew attention to the importance of PST inhibitors in limiting the accuracy of previous assay procedures [4]. The linear relationship found here was one piece of evidence that there was not significant inhibition of PST under these assay conditions. Other evidence is reviewed below.

PST activity for all three tissues also increased in a linear fashion with increasing incubation time for up to 40 min. An incubation time of 30 min was employed in all experiments.

Effects of pH. The effect of pH on PST activity was determined with four buffer systems: 16 mM sodium acetate, 8 mM potassium phosphate, 16 mM Tris–HCl, and 16 mM glycine–sodium hydroxide (Fig. 2). The concentrations of buffer described were those present in the final reaction mixtures. In all cases pH values were determined at room temperature in the presence of all components of the reaction mixture. The pH optima for liver, brain, and kidney with potassium phosphate buffer were approximately 7.0, 6.7, and 7.2 respectively. These values were those chosen for use in the final assay system and potassium phosphate was chosen as the buffer.

Effect of BSA and dithiothreitol. Because many enzymes are unstable when highly diluted in solutions of low protein concentration, PST activity was measured in the presence of various concentrations of BSA. Enzyme activity in rat liver, the most dilute of the preparations (1:80,000, v:v), increased 3-fold in the presence of 0.125% BSA. PST activity in homogenates of brain and kidney increased approximately 20% in the presence of 0.125% BSA. The enzyme activities of all homogenates decreased at BSA concentrations greater than 0.25%. Therefore, all assays were performed with tissue homogenates diluted with 0.125% BSA.

The activities of many conjugating enzymes, including partially purified PST, have been reported to increase in the presence of sulfhydryl reducing reagents such as dithiothreitol (DTT) [14, 15]. Therefore, PST activity was measured in the presence of five concentrations of DTT ranging from 1 to 16 mM. DTT had little effect on brain PST activity, but the liver and kidney enzyme activities increased

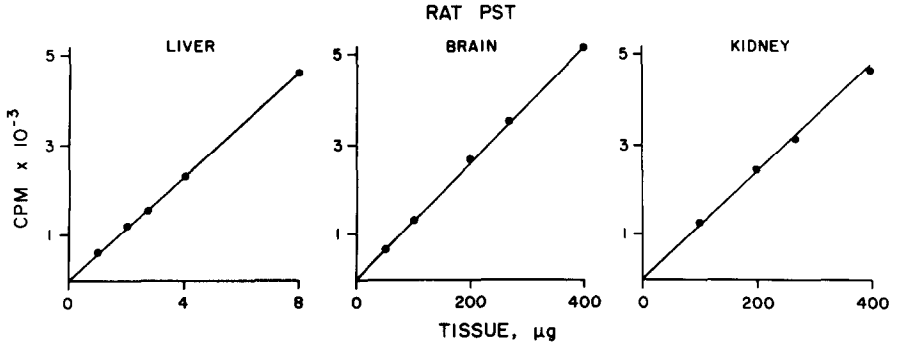

Fig. 1. Effect of increasing tissue concentration on PST activity. The abscissa indicates μg of tissue per reaction tube. Each value is the mean of three determinations.

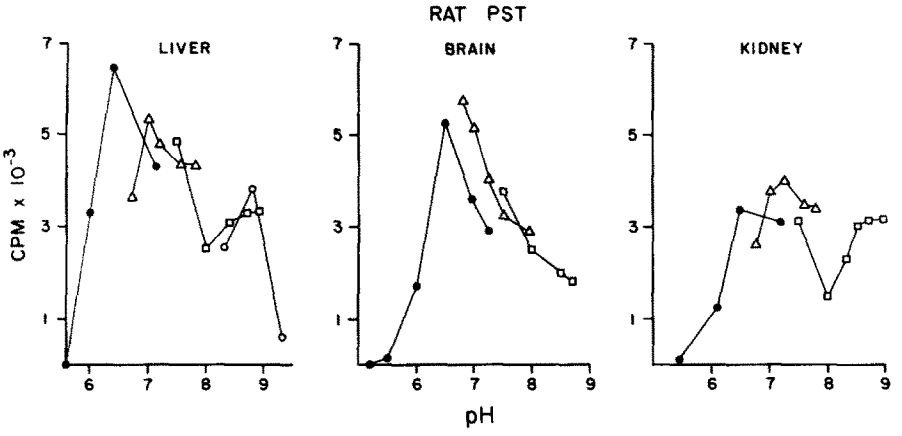

Fig. 2. Effect of pH on PST activity. Each value is the mean of three determinations with the following buffer systems: sodium acetate (●——●); potassium phosphate (△——△); Tris-HCl (□——□); and glycine-NaOH (○——○).

5- and 3-fold, respectively, in the presence of 8 mM DTT. This concentration of DTT was used in all reaction mixtures.

Effect of substrate concentration. PST activities in rat liver, brain, and kidney homogenates were measured in the presence of various concentrations of the two substrates for the reaction, MHPG and PAPS. The results obtained with MHPG are shown in Fig. 3. PST activity decreased at MHPG concentrations above 0.125 mM in brain and above 0.5 mM in liver and kidney. Apparent K_m values for MHPG were 0.15, 0.14 and 0.02 mM for liver, kidney, and brain respectively, estimated by the method of Wilkinson. When estimated by the method of Eisenthal and Cornish-Bowden the values were 0.13, 0.14 and 0.02 mM for liver, kidney, and brain respectively.

The effect of PAPS concentration on PST activity was also determined. Six different concentrations of PAPS ranging from 0.05 to 1.6 μM were tested. Apparent K_m values for PAPS calculated from these data were: liver, 0.11 μM; kidney, 0.07 μM; and brain, 0.07 μM when estimated by the method of Wilkinson. Values estimated by the method of Eisenthal and Cornish-Bowden were 0.15 μM for liver, 0.07 μM for kidney, and 0.08 μM for brain. A final concentration of 0.4 μM [^{35}S]PAPS was used in all experiments even though it did not result in saturation of the enzyme. This was done because the counts per minute in blank values rose sharply at higher PAPS concentrations and reduced the sensitivity of the assay. In addition, the cost of the radiolabeled sulfur donor made the use of higher concentrations prohibitively expensive. Other investigators have used subsaturating PAPS concentrations to assay PST activity for similar reasons [9].

Identification of reaction product. The product of the reaction was identified by thin-layer chromatography with two solvent systems; isopropyl alcohol–water–ammonium hydroxide (10:2:1), and isopropyl alcohol–*n*-butanol–water–formic acid (60:20:19:1). In both solvent systems over 95% of the radioactive product formed by all three preparations migrated with authentic MHPG sulfate.

Recovery of added PST activity. Although lack of inhibition of PST under these assay conditions was suggested by the linear increase in enzyme activity with increasing tissue quantity, additional experiments were performed to determine whether the effects of endogenous enzyme inhibitors had been negated. Partially purified rat liver PST was added to liver and kidney homogenates and partially purified rat brain PST was added to brain homogenates and the recovery of the exogenously added enzyme activity was measured. When recoveries of partially purified PST from the three tissues were expressed as percentages of the expected activities, these values

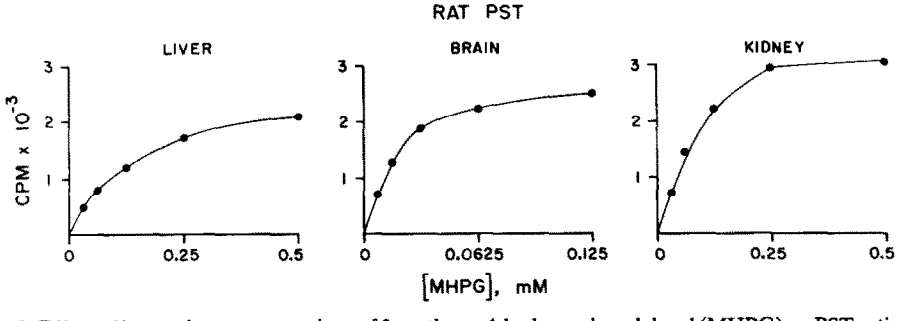

Fig. 3. Effect of increasing concentrations of 3-methoxy-4-hydroxyphenylglycol (MHPG) on PST activity. Each value is the mean of three determinations.

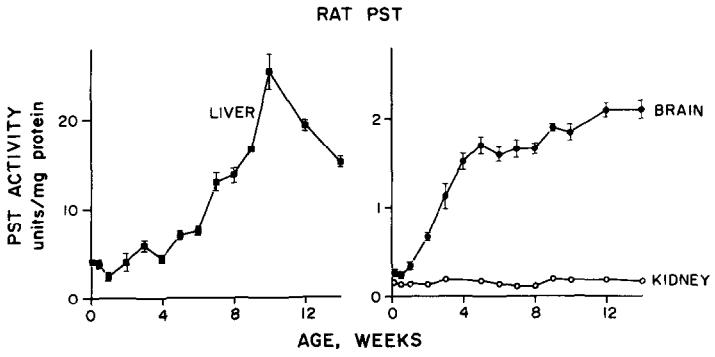

Fig. 4. Effect of growth and development on PST activity. Each value is the mean ± S.E.M. of determinations in three to five rats that varied from 1 day to 14 weeks of age.

were 89 ± 10, 89 ± 5 and 108 ± 10% (mean ± S.E.M., N = 5) for liver, brain, and kidney preparations respectively. These results supported the conclusion that the effects of endogenous PST inhibitors had been negated by the high degree of tissue dilution used under these assay conditions.

Effects of growth and development on rat PST activity

As a first step in the study of the regulation of rat PST activity, the effects of growth and development on the enzyme activity were measured. This was the first series of experiments performed because age-related changes in enzyme activity might confound attempts to study other regulatory mechanisms. PST activities were measured in liver, kidney, and brain of male Sprague–Dawley rats ranging from 1 day to 14 weeks of age (Fig. 4). When the results were expressed as units of enzyme activity per g tissue, there was a 4.1-fold increase in kidney, a 7.8-fold increase in liver, and a 7.7-fold increase in brain enzyme activity during that period of time. However, when the results were expressed as units of enzyme activity per mg protein (Fig. 4), there was no increase in renal activity, a 6.3-fold increase in liver activity at 10 weeks of age and a 6.6-fold increase in brain activity. Brain PST activity increased sharply between 1 and 4 weeks of age but the most rapid increase in liver enzyme activity occurred between 6 and 10 weeks of age (Fig. 4). A comparison of rat PST activities for liver, kidney and brain expressed both per g tissue and per mg protein obtained in a separate experiment is shown in Table 1.

Changes of PST activities in liver and brain during growth and development might have resulted from changes in levels of endogenous enzyme inhibitors, activators, or competing enzyme systems rather than from changes in PST activity itself. To test this possibility, partially purified liver and brain PST were added to homogenates of these two tissues from newborn animals. In both cases, the recoveries of partially purified enzyme activity were 89%, values very similar to those obtained with adult rat tissue homogenates. These results made it unlikely that changes in rat PST activity with age resulted from changes in the levels of enzyme inhibitors, activators, or competing enzyme systems.

Table 1. Rat PST activities*

Organ	Activity	
	Units per g tissue	Units per mg protein
Brain	57.5 ± 5.7	1.91 ± 0.16
Liver	3098 ± 274	31.7 ± 3.1
Kidney	26.2 ± 2.1	0.56 ± 0.04

* Each value is the mean ± S.E.M. of five male rats 11–12 weeks of age.

Effect of glucocorticoids on rat PST activity

Effect of adrenalectomy and dexamethasone treatment on PST activity. In an attempt to study possible humoral regulation of PST activity, male Sprague–Dawley rats that had undergone either adrenalectomy or a sham-operation 1 week previously were injected subcutaneously daily with either saline or dexamethasone, 7 μmoles/kg. PST activities in brains, livers, and kidneys were measured 3, 24, and 72 hr after the initial injections (Fig. 5). There were no significant differences between enzyme activities in adrenalectomized and sham-operated animals. Brain PST activity did not change in response to dexamethasone treatment. Rat liver PST activity increased slightly after glucocorticoid treatment, but the increase was significant only after 72 hr in adrenalectomized animals ($P < 0.05$). However, rat kidney PST activity increased dramatically after 72 hr of daily injections of dexamethasone (Fig. 5). Adrenalectomized animals treated with dexamethasone daily for 72 hr had an average kidney PST activity of 2.19 ± 0.25 units/mg protein (mean ± S.E.M., N = 5) compared with an average activity of 0.45 ± 0.05 units/mg protein in adrenalectomized, saline-injected controls ($P < 0.01$). Sham-operated dexamethasone-treated animals had an average kidney PST activity of 2.46 ± 0.48 units/mg protein (mean ± S.E.M., N = 5), while sham-operated control rats injected with saline daily had an average activity of 0.51 ± 0.10 units/mg protein ($P < 0.01$).

To determine whether the increase in renal PST activity in response to dexamethasone might have been due to a direct effect of the drug on the enzyme, dexamethasone at a final concentration of 7×10^{-6} M was added to a high-speed supernatant fraction of kidney homogenate from a normal male Sprague–Dawley rat. This concentration of dexa-

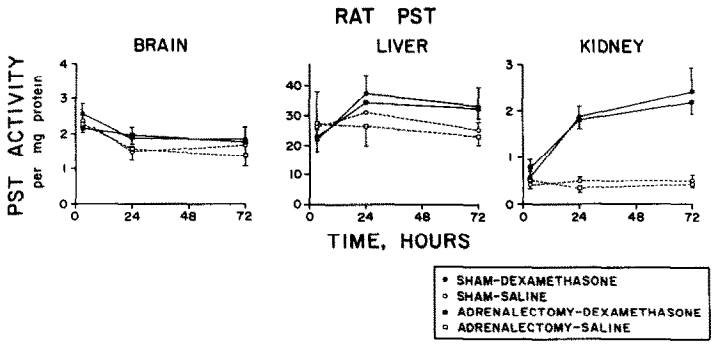

Fig. 5. Effects of adrenalectomy and dexamethasone on PST activity. Male Sprague–Dawley rats were subjected to adrenalectomy or sham-operation at 10 weeks of age. Animals were treated with dexamethasone, 7 μmoles/kg per day for 3 days. The abscissa represents the number of hours after the initial dexamethasone injection. Each value is the mean ± S.E.M. of determinations in five animals.

methasone represented the maximum level of drug to which the tissue might have been exposed if the entire subcutaneous dose had been completely absorbed instantaneously and had been evenly distributed throughout the animal. Direct exposure to a 7×10^{-6} M concentration of dexamethasone had no effect on PST activity. Enzyme activity assayed in the presence of dexamethasone was 16.3 ± 2.7 units/g tissue while that assayed in the absence of drug was 15.7 ± 2.4 units/g (mean ± S.E.M., N = 5).

The increase in rat kidney PST activity after dexamethasone treatment might have been due to changes in endogenous PST activators, inhibitors, or competing enzyme systems. To test this possibility pooled kidney supernatant fractions from five rats treated with dexamethasone for 48 hr and from five saline-treated control animals were assayed separately and in mixtures of 1:3, 1:1, and 3:1 (v:v). The enzyme activities in the mixtures were similar to those expected on the basis of direct additive contributions by each pooled homogenate (Table 2). This result made it unlikely that the 5-fold increase in rat kidney PST after treatment with dexamethasone was due to changes in tissue enzyme inhibitors, activators, or competing enzyme systems.

Table 2. Results of mixing pooled high-speed kidney supernatant fractions from dexamethasone-treated rats with pooled supernatant fractions from control rats*

Enzyme source		PST activity Observed	Expected
Dexamethasone-treated rats		4208	
Mixtures			
(High:low)	(3:1)	3265	3357
	(1:1)	2641	2505
	(1:3)	1715	1654
Control rats		802	

* PST activities are expressed as counts per minute. Observed values represent the average of three determinations. Expected values were calculated on the basis of simple additive contributions from each of the two pools.

Response of kidney PST to increasing doses of dexamethasone. The dose–response relation of kidney PST activity after treatment with dexamethasone was determined (Fig. 6). All rats were injected daily for 2 days and were killed 48 hr after the initial injection. Kidney PST activity increased from a con-

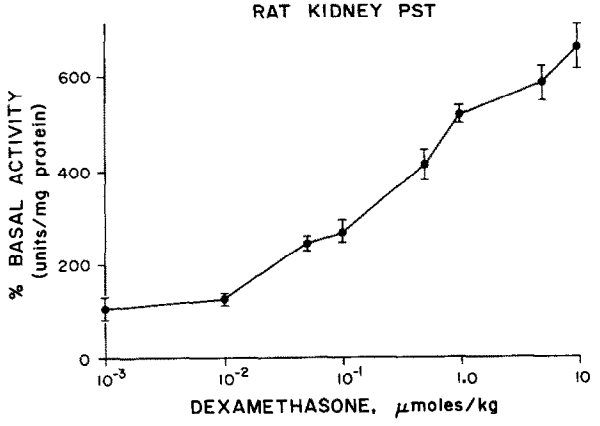

Fig. 6. Effect of various doses of dexamethasone on rat kidney PST activity. Male Sprague–Dawley rats were treated daily for 2 days with the indicated doses of dexamethasone. Each value is the mean ± S.E.M. of determinations in five animals.

trol level of 0.56 ± 0.04 units/mg protein (mean ± S.E.M., N = 5) to 1.38 ± 0.08 units/mg protein in response to a dose of dexamethasone as low as 0.05 μmole/kg per day and to 3.69 ± 0.25 units/mg protein in response to a dose of 10 μmoles/kg per day ($P < 0.001$).

Effect of protein synthesis inhibition on PST response to dexamethasone. To determine whether the increase in kidney PST activity after dexamethasone treatment was dependent on protein synthesis, rats were treated with a protein synthesis inhibitor. The animals were treated with cycloheximide, 0.9 mg/kg, subcutaneously 2 hr prior to the injection of 1 μmole/kg of dexamethasone. The cycloheximide injections were repeated at 6-hr intervals for 24 hr. This dosage schedule for cycloheximide has been shown to inhibit [^{3}H]leucine incorporation into rat protein by about 70% [16]. Control animals received saline alone, cycloheximide alone, or dexamethasone alone (Fig. 7). Kidney PST activity was 0.59 ± 0.05 units/mg protein in saline-injected controls and rose to 1.70 ± 0.08 units/mg protein (mean ± S.E.M., N = 5) in rats treated with dexamethasone (Fig. 7). There was no significant difference between the average renal PST activities in rats treated with saline and animals that had received only cycloheximide. Rats that had received both dexamethasone and cycloheximide had an average kidney enzyme activity of 0.78 ± 0.12 units/mg protein (mean ± S.E.M., N = 5, $P < 0.001$ versus dexamethasone alone). Cycloheximide blocked 83% of the anticipated increase in renal PST activity after treatment with dexamethasone.

Dexamethasone effect in a variety of rat strains. Eight inbred and two outbred rat strains were studied to determine whether there were strain variations, variations which might be genetic in origin, in the effect of dexamethasone on rat brain, liver, and kidney PST activities. All strains were treated daily for 2 days with 1 μmole/kg of dexamethasone. The dose–response curve (Fig. 6) indicated that this dose would provide a near maximum response. Higher doses were not used because one goal of the experiment was to determine whether some strains might be relatively resistant to dexamethasone-induced increases in the renal enzyme activity. Such "non-responsive" strains would have been of interest in biochemical genetic experiments. Rat brain PST activity in all strains was not changed significantly by dexamethasone treatment (Fig. 8). Rat liver PST in all strains showed small increases in response to dexamethasone. The increase in hepatic PST activity varied from 14.1 ± 4.6% (mean ± S.E.M.) in Lewis rats to 64.6 ± 3.4% in Wistar–Kyoto animals (Fig. 8). Kidney PST activity in all strains increased sharply after dexamethasone treatment. The increases in renal activity varied from 123.3 ± 1.8% in ACI rats to 417.4 ± 18.3% in Sprague–Dawley animals (Fig. 8).

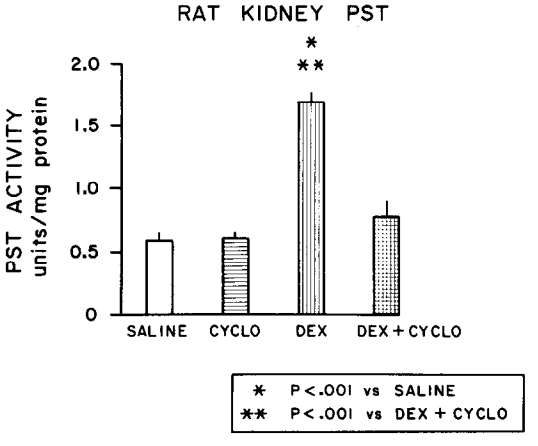

Fig. 7. Effect of cycloheximide on the response of rat kidney PST activity to dexamethasone. Each value is the mean ± S.E.M. of determinations in five animals. CYCLO represents treatment with cycloheximide, 0.9 mg/kg every 6 hr for 24 hr, and DEX represents treatment with dexamethasone, 1 μmolc/kg.

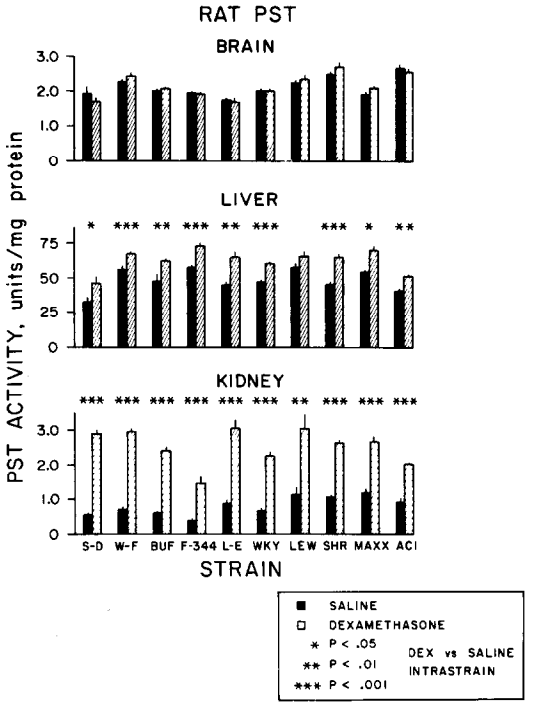

Fig. 8. Effect of dexamethasone on PST activity in ten rat strains. Animals received 1 μmole/kg of dexamethasone daily for 2 days. Each value is the mean ± S.E.M. of determinations in five rats. Abbreviations of strain names include the following: S-D, Sprague–Dawley; W–F, Wistar–Furth; BUF, Buffalo; F- 344, Fischer-344; L–E, Long–Evans; WKY, Wistar–Kyoto; LEW, Lewis; SHR, spontaneously hypertensive rat of Okamoto.

DISCUSSION

PST plays an important role in the metabolism of monoamines, monoamine metabolites, and phenolic drugs [1–3]. The regulation of PST is not well understood, in part because of the existence of potent endogenous PST inhibitors which make it difficult to assay the enzyme activity in tissue homogenates [4]. The series of experiments described above included determination of conditions for the assay of PST activity in highly diluted tissue homogenates, conditions that negated the effects of endogenous

inhibitors. The assay was used to study the effects of growth and development and of exogenous glucocorticoids on rat PST activity. Large increases in liver and brain enzyme activities occurred during growth and development. Although adrenalectomy had little effect on PST activity in Sprague–Dawley rats, administration of the synthetic glucocorticoid, dexamethasone, resulted in a large increase in kidney and a smaller increase in liver enzyme activity. The ability of cycloheximide to block the increase in renal PST activity in response to dexamethasone indicated that protein synthesis was necessary for the elevation in enzyme activity to occur. However, these results did not necessarily show that the effect of cycloheximide resulted from a direct inhibition of the synthesis of PST itself. Finally, it was demonstrated that the response of rat kidney PST to dexamethasone was not unique to Sprague–Dawley rats, but occurred in each of the ten separate rat strains studied. Whether endogenous glucocorticoids might play a role in the physiologic regulation of PST in the rat kidney and liver remains to be determined.

The biochemical properties of PST in the rat have been the subject of both controversy and confusion. This situation may result in part from the use of a variety of assay conditions and substrates to study the enzyme as well as the comparison of data obtained with tissue homogenates with that obtained with enzyme preparations at various states of purification [9, 14, 15, 17, 18]. It might also result from the existence of multiple forms of enzyme—forms with somewhat differing properties and differing substrate specificities [17, 19]. The results of the experiments described here apply only to enzyme activity or activities capable of catalyzing the sulfate conjugation of MHPG, and alterations in the activities of forms of PST catalyzing the sulfate conjugation of other substrates would not be detected by this assay procedure. However, valuable information about the properties of rat PST can be gained from studies of both the purified enzyme and measurements of enzyme activity in tissue homogenates or partially purified preparations. The results of these two approaches are complementary. The findings described here represent a significant step toward increasing our understanding of the regulation of PST activity in an experimental animal model and will provide a basis for future studies of the regulation of this important drug and neurotransmitter-metabolizing enzyme activity.

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