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Characterization and post-natal development of rat cerebellum tyrosylprotein sulfotransferase

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Tyrosine sulfation has been demonstrated to be a widespread post-translational modification of a variety of functionally diverse, biologically active peptides and proteins [1, 2]. For several of the identified tyrosine sulfated proteins and peptides, such as cholecystokinin (CCK*) [3], gastrin [4], phyllokinin [5], leukosulfakinin [6] and C4 of complement [7], sulfation has been shown to result in the optimization of biological activity.

Sulfoconjugation of the tyrosyl residues of proteins and peptides has been demonstrated to be catalyzed primarily within the Golgi apparatus by the enzyme, tyrosylprotein sulfotransferase (TPST) [8, 9]. This enzyme utilizes 3′-phosphoadenosine-5′-phosphosulfate (PAPS) as the sulfate donor and requires that the acceptor tyrosine residue be surrounded by a highly acidic amino acid domain within the protein [8, 10]. Using the acidic, synthetic polymer, poly Glu_6,Ala_3,Tyr_1 (EAY), we have demonstrated recently that TPST displays a fairly wide tissue distribution in the rat [9]. Of the tissues examined, rat liver possesses the highest specific activity, more than 2.5-fold higher than that in the other tissues examined. TPST activity also displays a variable distribution in rat brain, with the cerebellum and pituitary containing the highest specific activity.

Little is known about the factors that influence or promote tyrosine sulfation of peptides and proteins within the brain and other tissues. For example, one such factor that has not been addressed is the post-natal development of TPST, as well as whether the formation of this enzyme during development correlates with the synthesis of biologically active tyrosine sulfated proteins and peptides. In this study, we begin to address these latter questions by observing post-natal developmental changes of TPST activity in both rat cerebellum and liver.

EAY (average mol. wt 47,000), MES buffer, Lubrol-PX and unlabeled PAPS were purchased from the Sigma Chemical Co. (St Louis, MO), and bicinchoninic acid protein assay solution was obtained from Pierce (Rockford, IL). [^{35}S]-5′-PAPS (0.6 to 1.0 Ci/mmol) was purchased from New England Nuclear (Boston, MA). All other chemicals used were the purest available from commercial sources.

Timed pregnant rats were obtained from Holtzman Laboratory Animals (Madison, WI) and housed indi-

* Abbreviations: CCK, cholecystokinin; TPST, tyrosylprotein sulfotransferase; PAPS, 3′-phosphoadenosine-5′-phosphosulfate; EAY, polymer of Glu_6,Ala_3,Tyr_1; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; and MES, a-(*N*-morpholine)ethanesulfonic acid.

vidually. The date of birth was considered to be day 0 and all pups used in this study were born within 16 hr of one another. After birth, pups were removed from their mothers, pooled, and randomly replaced in litters of eight per mother. A total of six to eight male and female pups were used at each time point. The rats were killed by decapitation, and their cerebellums and livers were removed rapidly. The tissues were then homogenized by a motorized glass/Teflon homogenizer in 5 vol. of cold 0.32 M sucrose and centrifuged at 1000 *g* for 10 min. The supernatant solutions were removed and placed on ice, and the pellets were resuspended in ice-cold 0.32 M sucrose (one-half the homogenization volume) and recentrifuged at 1000 *g* for 10 min. The resulting supernatant fractions were combined with those from the previous step, and the combined solutions were centrifuged at 10,000 *g* for 10 min. The 10,000 *g* supernatant solutions were used as the enzyme source unless otherwise specified.

TPST activity of rat cerebellum and liver was assayed by the method of Rens-Domiano and Roth [9]. Briefly, the standard mixture consisted of 20 mM $MnCl_2$, 50 mM NaF, 1 mM 5′-AMP, 0.5% (w/v) Lubrol PX, 2 μM [^{35}S]PAPS (~2000 dpm/pmol), 2 μM EAY and an appropriate enzyme aliquot, all in a final volume of 100 μl of 40 mM MES buffer, pH 6.5. To optimize the measurement of cerebellar TPST activity, 0.1 M NaCl was also included in the reaction mixture (see results below).The assay mixture was incubated at 30° for 30 min and the reaction stopped by acid precipitation of the ^{35}S-labeled polymer onto a 2.0 × 2.5 cm 3 MM Whatman filter paper. The filter paper was then washed three times in a cold 10% trichloroacetic acid/10 mM Na_2SO_4 bath, dried, and the radioactive product precipitated on the filter paper was measured by liquid scintillation chromatography. All reactions were linear with respect to time and protein concentration. Protein concentrations were determined by a bicinchoninic acid protein assay method [11].

As illustrated in Fig. 1, the optimal pH value for the sulfation of EAY by rat cerebellar TPST was approximately 6.7. This value is similar to the pH optimum reported previously for the sulfation of EAY by rat liver TPST [9].

Based on prior studies of other rat brain sulfotransferases, it was speculated that NaCl may stimulate cerebellar membrane-bound sulfotransferase activity [12, 13]. The data shown in Fig. 2. demonstrate that NaCl also potentiated rat cerebellar TPST activity. Incubations, using an enzyme fraction prepared from a 9-day-old rat, performed in the absence of NaCl resulted in the formation of only 0.05 pmol/min/mg of EAY-SO_4, whereas addition of either 0.05 or 0.1 M NaCl to the incubation mixture resulted in approximately a 4.5-fold increase in EAY sulfation. Higher concentrations of salt were found to decrease this optimal activity. Similar results were observed when the cerebellum from an adult rat was used as the enzyme source. Accordingly, to maximize TPST activity in the cerebellum, assays were performed in the presence of 0.1 M NaCl. While not shown, rat liver TPST activity did not display a similar activation, at all ages examined, and, therefore, liver TPST assays were performed in the absence of NaCl.

The post-natal changes in rat cerebellum and liver TPST sulfation of the synthetic acidic polymer, EAY, are shown in Fig. 3. As the data reveal, the developmental patterns of TPST activity observed in the rat cerebellum and liver were considerably different. At day 1 after parturition, TPST activity was measurable in both tissue extracts, with the specific activity in the cerebellum (0.06 pmol/min/mg) being about half that found in the liver (0.13 pmol/min/mg). Cerebellum TPST activity increased steadily from day 1 to a maximal plateau level of 0.15 pmol/min/mg between

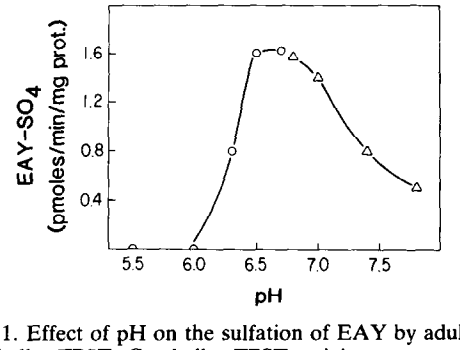

Fig. 1. Effect of pH on the sulfation of EAY by adult rat cerebellar TPST. Cerebellar TPST activity was measured as described in the text in a microsomal pellet prepared by centrifuging the 10,000 *g* supernatant fraction for 100,000 *g* for 60 min in the presence of 20 mM MES (○) or 20 mM HEPES (△) buffer solutions.

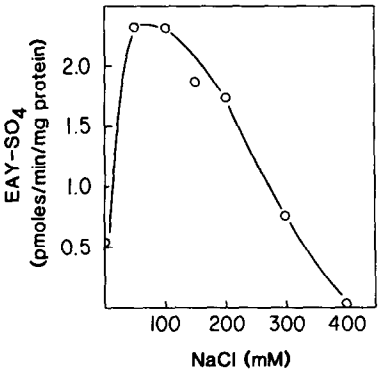

Fig. 2. Effect of NaCl on the sulfation of EAY (2 μM) by cerebellar TPST. TPST activity was measured in the 10,000 *g* supernatant fraction prepared from 9-day-old rat pups.

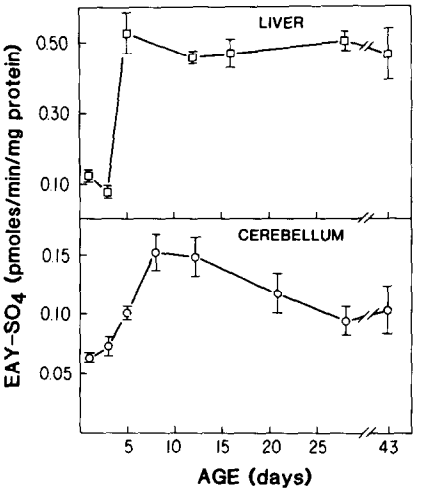

Fig. 3. Development of TPST sulfation of EAY (2 μM) in the rat. Liver (□) and cerebellar (○) TPST were measured as described in the text, using the 10,000 *g* supernatant fraction as the enzyme source. Each point represents the mean sulfotransferase activity from six to eight pups ± SE.

days 8 and 12. Beyond day 12, cerebellar TPST activity began to decline slowly until reaching adult levels of 0.10 pmol/min/mg. In contrast, rat liver TPST activity decreased slightly from day 1 to day 3, only to increase 8-fold to adult levels of 0.48 pmol/min/mg by day 5. In neither the rat liver nor the cerebellum were there any significant differences observed between the post-natal development of TPST activity in male and female rat pups.

The data presented in this paper suggest that rat TPST activity undergoes post-natal developmental changes and that there are significant differences between the development of EAY sulfation within the liver and the cerebellum. Since TPST is involved in the post-translational modification of a variety of proteins and peptides, these developmental changes in sulfotransferase activity can have a profound effect on the chronological appearance of these essential biologically active agents. These data may explain the differences reported in the variation in the sulfation of gastrin at different sites of synthesis during development [4, 14]. In this regard, it has also been demonstrated that, during rat brain development, the tyrosine-sulfated peptide hormone, CCK-8, undergoes regional variations [15]. Interestingly, there appears to be similarities in the pattern of development of CCK-like immunoreactivity in the cerebellum and the development of TPST activity in this brain region. Both appear to reach a maximal post-natal plateau of activity around days 8–12 and subsequently decrease to attain adult levels at about 3 weeks of age.

As mentioned previously, TPST has been reported to be located within the Golgi apparatus membrane [8, 9]. In the cerebellum, the optimal activity of another Golgi sulfotransferase, galactoceramide sulfotransferase [16, 17], has been found to occur between day 18 and day 20 after parturition, and that this time period corresponds to the period of maximal myelination. Thus, the developmental pattern of rat cerebellar TPST, as demonstrated herein, is different from that previously reported for galactoceramide sulfotransferase. One possible explanation for this difference in the development of two Golgi enzymes in the cerebellum is that these sulfotransferases are localized within different cerebellar cell types, although other reasons for this discrepancy are plausible. Nevertheless, these data suggest that the appearance of both TPST and galactoceramide sulfotransferase in the cerebellum is regulated based on their functional requirements during development.

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