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OCCURRENCE AND DISTRIBUTION OF SEROTONIN-*O*-SULFOTRANSFERASE IN HUMAN BRAIN; A NEW RADIOISOTOPIC ASSAY

HIROYOSHI HIDAKA AND JAMES AUSTIN*

*Institute of Biochemistry, Faculty of Medicine, University of Nagoya, Nagoya (Japan) and *Division of Neurology, University of Colorado Medical Center, 4200 E. 9th Ave., Denver, Colo. 80220 (U.S.A.)*

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SUMMARY

The formation of serotonin-*O*-sulfate by a sulfotransferase of human brain was demonstrated using 3'-phosphoadenosine-5'-phospho[^{35}S]sulfate ([^{35}S]PAPS) as sulfate donor. A new radioisotopic assay of the sulfotransferase was also developed. Serotonin-*O*-[^{35}S]sulfate, formed enzymatically, was estimated in a liquid scintillation counter after unreacted [^{35}S]PAPS and [^{35}S]sulfate were removed by a column of Biorex 4 resin. Serotonin sulfotransferase activity was measured in various regions of the human brain. The highest activity was found in the hypothalamus, the lowest in the thalamus.

INTRODUCTION

It has been suggested that serotonin becomes physiologically inert through enzymatic O-sulfation[1].

An enzyme catalyzing the transfer of sulfate from 3'-phosphoadenyl phosphosulfate to serotonin has recently been purified from rabbit liver and brain by HIDAKA *et al.*[2,3].

The presence of this enzyme in human brain could be important in regulating the physiological action of serotonin. The purposes of this communication are to present the distribution of this enzyme in human brain, to note a new radioisotopic method for assaying this enzyme, and to report the effect of certain compounds on the activity of the enzyme.

MATERIALS AND METHODS

Normal adult human brain was obtained at autopsy 5 h after death. The 53-year-old man died suddenly of an acute myocardial infarction. Specified regions

Abbreviations: [^{35}S]PAPS, 3'-phosphoadenosine-5'-phospho[^{35}S]sulfate; PCMB, *p*-chloromercuribenzoate.

of the brain were quickly dissected out in the cold room and were homogenized in 3 vol. of cold phosphate buffer (pH 7.4, 0.05 M) using a Polytron homogenizer. Rabbit brains were removed immediately after sacrifice by decapitation and were homogenized with 3 vol. of cold buffer. Authentic serotonin-*O*-sulfate was synthesized according to KISHIMOTO *et al.*[4]. 3'-Phosphoadenosine-5'-phospho[^{35}S]sulfate ([^{35}S]-PAPS) was obtained commercially from the New England Nuclear Corp. or was kindly supplied by Dr. Bimal Bachhawat. Biorex 4 resin (Cl^- type, 200–400 mesh) was obtained from Biorex Co. Ltd. All other chemicals were obtained commercially.

Each ml of the enzyme assay system contained: 0.1 mM of serotinin creatine sulfate, 0.01 μC of [^{35}S]PAPS, 0.1 M of phosphate buffer (pH 7.4), and distilled water to make 1.0 ml. Serotonin sulfotransferase was partially purified from brain according to HIDAKA *et al.*[3]. Two fractions were used as enzyme solutions. One was the fraction which precipitated when an equal volume of saturated $(NH_4)_2SO_4$ solution was added. The other was a high speed supernatant (105 000 $\times$ *g* for 90 min). Each enzyme solution was dialyzed against 0.0167 M pyrophosphate–H_2SO_4 buffer and then stored at $-30°$.

The product of this assay procedure was identified as serotonin-*O*-[^{35}S]sulfate both by paper chromatography and by paper electrophoresis using procedures previously described[5]. The effluent which passed through a (0.5 cm $\times$ 3 cm) column of Biorex 4 (Cl^- form, 200–400 mesh) was evaporated under N_2 at room temperature. The residue was dissolved in 1 ml of distilled water, and 50 μl of the solution was applied to Whatman No. 2 paper. Ascending paper chromatography was carried out using butanol–acetic acid–water (4:1:1, by vol.), 20% KCl, isopropanol–6 M ammonia, (20:3, by vol.), or butanol–pyridine–water (1:1:1, by vol.), as solvent systems. Electrophoresis was carried out using 120 V (3.3 V per cm) in 0.1 M citrate buffer (pH 5.5). Kodak X-ray film was used for detecting radioactive spots. The paper strip and the X-ray film were placed together in a box in the dark room for one week. ^{35}S-containing substances on the paper were analysed by previously described methods[5].

RESULTS

A new radioisotopic assay method for serotonin sulfotransferase

Rabbit brain was used as the standard enzyme source because of its high activity[3]. Several anion exchange resins were examined for their ability to separate the authentic serotonin-*O*-sulfate from contaminating [^{35}S]PAPS and [^{35}S]sulfate. Of these, Biorex 4 (Cl^-) was found most effective. This resin was found to take up [^{35}S]PAPS but not serotonin-*O*-sulfate under acidic conditions (0.1 M HCl). The reaction was started by adding the sulfotransferase. Incubations were then carried out at 37° with a mechanical shaker. Following incubation, the mixture was heated in boiling water for 2 min and mixed with 2 ml of 0.1 M HCl. Denatured protein was centrifuged off. The supernatant was then passed through a small column of resin to remove unreacted [^{35}S]PAPS and free ^{35}S. For this purpose, the resin had been washed before use with 20 ml of 0.1 M HCl and distilled water.

The column was then washed with 10 ml of M HCl and the total effluent (13 ml) was collected. An aliquot of this effluent (0.1–1.0 ml) was transferred to a scintillation vial containing 15 ml of Bray's solution. Radioactivity was then determined in a

liquid scintillation counter. As shown in Table I, high radioactivities were found only when the complete system was used. Fig. 1 shows the rate of enzymatic formation of the radioactive product and its dependence on enzyme concentration.

TABLE I

THE EFFECT OF OMITTING CERTAIN COMPONENTS OF THE REACTION MIXTURE ON THE FORMATION OF SEROTONIN-*O*-[^{35}S]SULFATE

Incubations were carried out at 37° for 30 min. 0.8 ml of column effluent was transferred to the scintillation vial which contained 15 ml of Bray's solution. A high speed supernatant (105 000 × *g* supernatant) was used as the enzyme source.

System	*Counts/min**	*Counts/min***
Complete	3450	7090
Minus serotonin	146	138
Minus [^{35}S]PAPS	40	48
Minus enzyme	100	120
Boiled enzyme	160	152

* 10 mg of whole brain tissue of rabbit.
** 20 mg of whole brain tissue of rabbit.

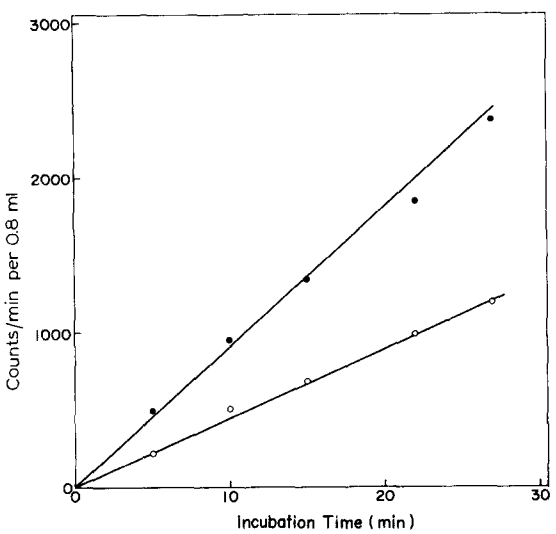

Fig. 1. Enzymatic formation of serotonin-*O*-sulfate as a function of time and enzyme concentration. The enzyme fraction used was precipitated by $(NH_4)_2SO_4$. Incubations were carried out at 37°: 0.1 mg or 0.05 mg of enzyme protein were used.

Identification of the product

The specificity of this assay for serotonin sulfotransferase was examined by comparing the radioactive product with authentic serotonin-*O*-sulfate.

The residue of each effluent was evaporated and dissolved in 1 ml of distilled water as described above. 50 μl of this solution was applied to paper and separated by chromatography or electrophoresis. The radioactive spots on film were compared

with spots on paper strips which had been treated with Ehrlich's reagent, α-nitroso-β-naphthol reagent, or ninhydrin.

The radioactive spot found after either electrophoresis or chromatography coincided with the pink spot found with Ehrlich's reagent but not with the blue spot of serotonin. The radioactive pink spot cochromatographed in four different solvent systems with authentic serotonin-*O*-sulfate. The R_F values were the same as noted elsewhere[5]. Moreover, the product was not separated from the authentic sample when the two were mixed together before paper chromatography or electrophoresis. The radioactive product turned purple with the ninhydrin reagent but developed no color with the α-nitroso-β-naphthol reagent. These results indicate that the compound contains an indole ring, an amino group, [^{35}S]sulfate, and no free hydroxyl group at the 5-position.

Effect of various compounds on the activity of the sulfotransferase

The presence of an active SH function in the enzyme was suggested by the increase of activity with cysteine or dithioerythritol (Table II). Therefore, the effect of adding *p*-chloromercuribenzoic acid (PCMB) on the activity of the enzyme was checked. PCMB was found to inhibit the activity 50% at $2.5 \cdot 10^{-5}$ M (Table II). In these respects the enzyme resembles phenol sulfotransferase.

Higher concentrations of Mg^{2+} or Mn^{2+} stimulated activity (Table II). It was of interest that pargyline, a potent inhibitor of monoamine oxidase, had no effect on the activity *in vitro* (Table II).

TABLE II

THE EFFECT OF VARIOUS COMPOUNDS ON THE ACTIVITY OF SEROTONIN SULFOTRANSFERASE

The fraction precipitated by $(NH_4)_2SO_4$ was used as the enzyme source. The assay system contained 0.1 mg of enzyme protein. Incubations were at 37° for 10 min.

Compound added	*Counts/min*	*% of change*
None (control)	1190	
Cysteine (10^{-3} M)	1680	+ 41
Dithioerythritol		
(10^{-2} M)	1700	+ 43
(10^{-3} M)	1500	+ 26
$MgCl_2$ ($5 \cdot 10^{-3}$ M)	1900	+ 60
($5 \cdot 10^{-4}$ M)	1200	0
$MnCl_2$ ($5 \cdot 10^{-3}$ M)	1460	+ 23
($5 \cdot 10^{-4}$ M)	1180	0
Pargyline ($1 \cdot 10^{-3}$)	1170	0
($5 \cdot 10^{-4}$)	1210	0
PCMB ($1 \cdot 10^{-4}$)	0	−100
($2.5 \cdot 10^{-5}$)	590	− 50
($5 \cdot 10^{-5}$)	900	− 25

Formation and distribution of serotonin O-sulfotransferase in human brain

The distribution of activity is given in Table III. The highest activity was found in the region of the hypothalamus. The lowest activity was found in the region of the thalamus.

TABLE III

DISTRIBUTION OF SEROTONIN SULFOTRANSFERASE IN HUMAN BRAIN

The high speed supernatant of human brain homogenate was used 0.4 ml of this supernatant was added to reaction mixture. Incubations were at 37° for 30 min.

Site	*Counts/min per 100 mg of tissue*
Hypothalamus	2054
Pons	1422
Caudate nucleus	1271
Cerebellum	1139
Putamen	1110
Hippocampus	1063
Thalamus	788

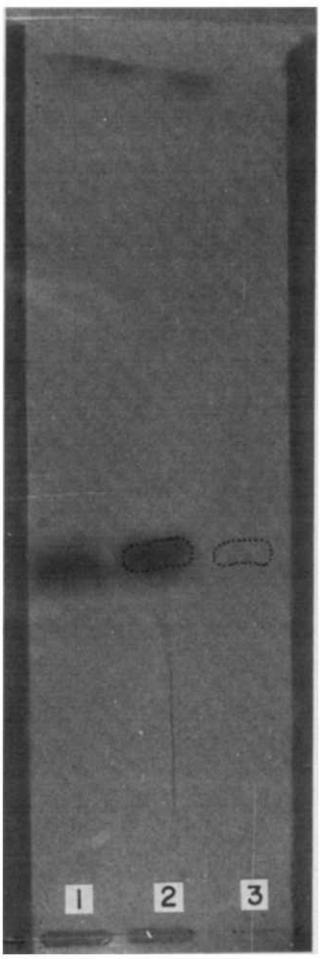

Fig. 2. Demonstration of formation of serotonin-*O*-sulfate by human brain (hypothalamus) using paper chromatography (20% KCl). Circles described by broken line indicate spots which turn pink when treated with Ehrlich's reagent. Dark spots indicate radioactivity. The method used is described in the text. (1) Product, (2) mixture of product and serotonin-*O*-sulfate, (3) serotonin-*O*-sulfate.

The formation of serotonin-*O*-sulfate by human brain was demonstrated using the hypothalamus. A typical result is shown in Fig. 2.

DISCUSSION

This report is the first demonstrating the occurrence of serotonin sulfotransferase in human brain. This enzyme has been found in the brain in the rabbit, dog, rat and cow by HIDAKA *et al.*[3]. The activity in human hypothalamus was only about one fifteenth that found in the whole fresh rabbit brain. The fact that the human brain was assayed perhaps 9 h after death may have influenced this result. The enzyme is, in general, a labile enzyme. Particularly after 1 week its activity decreased even when stored at −30°.

In our previous paper[6], a simple assay of serotonin sulfotransferase activity was presented. However, the assay system contained several enzyme systems which include both PAPS generating enzymes and sulfotransferases. Because these other enzymes existed in the assay system[9], inhibitory studies or precise kinetic studies could not be done.

Using the previous assay method, the K_m of the rabbit enzyme was 10 times higher than that of monoamine oxidase. We believe the sulfotransferase could help inactivate serotonin under conditions when serotonin is increased in tissues *in vivo*. For example, huge amounts of serotonin-*O*-sulfate are excreted in urine in patients who have a carcinoid tumor[7]. It is of note that serotonin-*O*-sulfate is found normally in the urine of man[8]. Some properties of serotonin sulfotransferase are similar to those of phenol sulfotransferase (*e.g.* stimulation by cysteine, dithioerythritol, or inhibition by PCMB), but the stimulation by Mg^{2+} is unique. Further investigations of these parameters appear indicated.

KORF AND SEBENS[9] observed no significant increase in the amount of serotonin-*O*-sulfate in rat brain following exposure to a monoamine oxidase inhibitor. As reported previously[3], rat brain contains relatively low enzyme activity.

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