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Note

Thin-layer chromatographic separation and quantitation of radioactively labelled 5-hydroxytryptamine, 5-hydroxytryptamine-O-sulfate, and 5-hydroxyindoleacetic acid

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Brain tissue and platelets contain appreciable quantities of a type of phenolsulfotransferase which *in vitro* catalyzes the transfer of inorganic sulfate from phosphoadenosine-phosphosulfate (PAPS) to the biogenic amines dopamine and 5-hydroxytryptamine (5-HT)[1–4]. Recent work has suggested that considerable quantities of the O-sulfate conjugates of these amines may be present in brain and platelets, but accurate identification and quantitation remain problematical[5–7]. To assist in the identification of 5-HT-O-sulfate 5-HT-O-SO_4 in aminergic tissue, we have developed a procedure for the chromatographic separation and quantitation of radioactively labelled 5-HT, 5-HT-O-SO_4, and 5-hydroxyindoleacetic acid (5-HIAA).

MATERIALS AND METHODS

Carrier solutions are prepared by dissolving 2 mg of each compound per ml of an isopropanol–10^{-3} *M* hydrochloric acid (1:1) mixture (complete dissolution generally requires at least 12 h at room temperature). 5-HT is used as the binoxalate salt (Sigma, St. Louis, MO, U.S.A.), 5-HIAA as the cyclohexylammonium salt (Calbiochem-Behring, La Jolla, CA, U.S.A.), and 5-HT-O-SO_4 as the 1/4 hydrate (obtained through the courtesy of Dr. Albert Manian, Neurosciences Research Branch, NIMH, Rockville, MD, U.S.A.). Silica gel plates (60 F_{254}) with a fluorescent indicator and either glass or aluminium backing can be used (Merck, Darmstadt, G.F.R., No. 5539-9H; obtained from Sargent-Welch, Skokie, IL, U.S.A., as catalogue No. S-18952-10-F). To prevent cross-contamination, lines are scored at 1.5-cm intervals, and the origin line is drawn with a lead pencil so as to lie at least 0.5 cm above the initial solvent level. Aliquots (2.5 μl) of each carrier solution are spotted for 1-cm distances along the origin, with a gently flowing stream of nitrogen gas used to dry each area immediately after the solution has been applied. In some cases, 2.5 μl (containing approximately 10,000 dpm) of a solution of [^{14}C]5-HT (58 mCi/mmole; Amersham, Arlington Hts, IL, U.S.A.) or [^{14}C]5-HIAA (15.2 mCi/mole; New

England Nuclear, Boston, MA, U.S.A.) may also be spotted at this time. After all the carriers and unknowns have been spotted, the plates may be wrapped with thin plastic sheeting (Saran Wrap; Dow Chemical, Midland, MI, U.S.A.) for protection from air oxidation prior to running the chromatography.

Tissue samples incubated with radiolabelled 5-HT are prepared for spotting by adding 100 μl of 0.4 *N* perchloric acid to the tissue pellets, vortexing, and then neutralizing with 5.6 μl of 7 *N* potassium hydroxide solution. Acid-precipitable material and $KClO_4$ are removed by brief centrifugation (1000 *g* for 5 min), and 5 μl of the clear supernatant are spotted on the plates by applying 2.5 μl at one time and drying under the nitrogen stream after each application.

Plates are placed in a tank which has been pre-equilibrated for 30 min with the solvent system of methyl acetate–isopropanol–58% ammonium hydroxide (45:23:10). The tank is sealed with vacuum grease and allowed to run for 1 h. Fresh solvent should be prepared for each run, since poor separation of 5-HT-O-SO_4 and 5-HIAA is achieved with solvent more than a few hours old, or if the same solvent is used for a second set of plates. After removal of the cnromatographed plate, the extent of solvent migration is marked. The plate is allowed to dry in air and examined under ultraviolet light. The carrier spots are circled with a No. 2 lead pencil and subsequently scraped into scintillation vials containing 10 ml of Aquasol (New England Nuclear).

RESULTS AND DISCUSSION

The typical spot separation observed under ultraviolet light is presented in Fig. 1. Fig. 2 delineates the distribution of radioactivity (obtained with a Varian Series

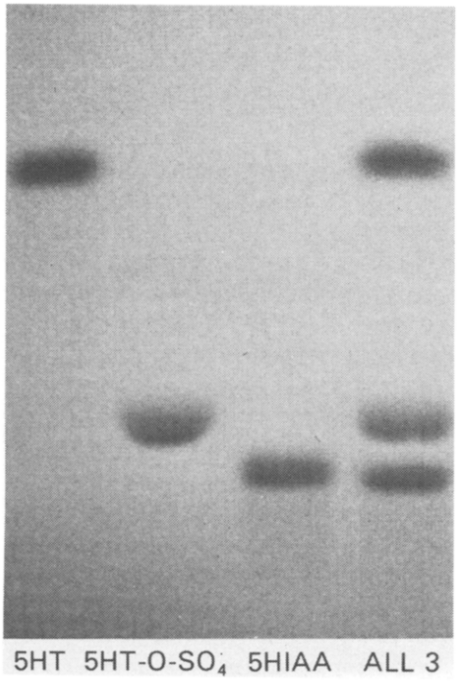

Fig. 1. Thin-layer chromatography plate spotted with either 5-HT, 5-HT-O-SO_4, 5-HIAA, or all three compounds, run as described in the text, and photographed under ultraviolet light.

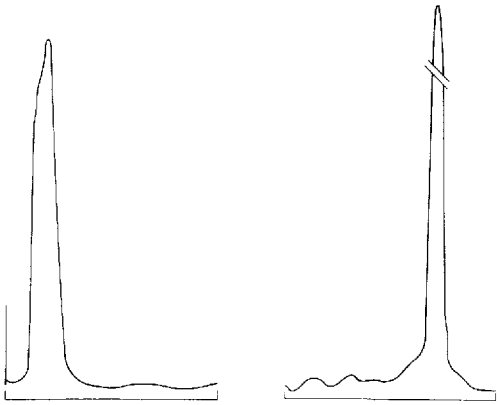

Fig. 2. Distribution of radioactivity on plates spotted with 10,000 dpm of either [^{14}C]5-HT (right) or [^{14}C]5-HIAA (left) and run as described in the text. The origin is represented by the vertical bar to the left of the baseline and the solvent front by the vertical bar to the right.

6000 thin-layer scanner; Varian Assoc., Palo Alto, CA, U.S.A.) along plates spotted with carriers plus either [^{14}C]5-HT or [^{14}C]5-HIAA. When [^{35}S]PAPS (1.3 Ci/mmole; New England Nuclear) was spotted with the three indole carriers, essentially 100% of the label remained at the origin (less than 0.5% in the 5-HT-O-SO_4 spot).

Both human platelets and brain tissue have been reported[1,3,4,7,8] to contain a phosphosulfotransferase which catalyzes the formation of 5-HT-O-SO_4. To test the ability of our system to detect the formation of the latter compound, we incubated washed human platelets and microsacs prepared from guinea pig cortex[9,10] for 30 min at 37°C with [^{3}H]5-HT, resuspended the tissues in fresh buffer[11], and incubated for 30 min at either 0°C or 37°C. Tissue was then pelleted and processed for thin-layer chromatography as described above. The results are presented in Table I, and suggest that both tissues contain appreciable amounts of labelled 5-HT-O-SO_4 after loading at 37°C followed by incubation at either 0°C or 37°C. As reported previously[12],

TABLE I

THIN-LAYER CHROMATOGRAPHIC SEPARATION OF LABELLED 5-HIAA, 5-HT-O-SO_4, AND 5-HT IN HUMAN PLATELETS AND GUINEA PIG CORTICAL MICROSACS PRELABELLED WITH [^{3}H]5-HT

Experimental procedure	*Percent of total dpm present at various spots on plate*			
	Origin	*5-HIAA*	*5-HT-O-SO_4*	*5-HT*
Human platelets				
30 min at 0°C	1.5%	0.8%	2.4%	95.4%
30 min at 37°C	0.8%	0.5%	1.7%	97.6%
Guinea pig cortical microsacs				
30 min at 0°C	0.7%	11.0%	5.0%	83.3%
30 min at 37°C	0.9%	19.2%	3.6%	76.2%

human platelets apparently contained no appreciable amounts of 5-HIAA regardless of the temperature of incubation. Microsacs contained almost two times more 5-HIAA when incubated at 37°C than when incubated at 0°C.

The method described here works well for whole brain tissue, microsacs, isolated microvessels[13], and platelets. When platelets are examined, the pellet can be mobilized with 100 μl of an isopropanol–10^{-3} *M* hydrochloric acid (1:1) mixture. With other tissues, however, use of this procedure resulted in plates with approximately 50% of the applied radioactivity remaining at the origin. Tissue-free supernates can be spotted directly, but produce better separation of the 5-HT-O-SO_4 and 5-HIAA spots when deproteinized first with 0.4 *N* perchloric acid and subsequently neutralized as described above.

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