School of Biological Sciences,
Flinders University of South Australia,
Bedford Park,
South Australia

B. S. TAY
R. McC. LILLEY
A. W. MURRAY
M. R. ATKINSON

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Simultaneous estimation of 5-hydroxytryptamine and 5-hydroxyindol-3-acetic acid in rat brain

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SEVERAL methods have been described for the separate estimation of tissular 5-hydroxytryptamine (5-HT) and 5-hydroxyindol-3-acetic acid (5-HIAA). According to the method of Bogdanski *et al.*[1] 5-HT is extracted into butanol and its native fluorescence measured after activation in 3 N HCl. Vanable[2] published a more specific and sensitive method in which ninhydrine is employed to form a fluorescent compound with 5-HT. The procedure described by Snyder[3] is based on the latter method. For the estimation of 5-HIAA several methods are based on extraction with diethyl-ether followed by quantitative estimation.[4, 5] Recently, Giacalone and Valzelli[6] proposed the use of butyl acetate for the extraction of the acid, followed by spectrofluorometric assay.

The method described below allows the simultaneous estimation of 5-HT and 5-HIAA in the same brain extract. It combines a slightly modified Snyder[3] procedure with the method of Giacalone and Valzelli[6] for the estimation of 5-HT and 5-HIAA respectively, and is based on the observation of Dreux,[7] who, in the course of urinary 5-HT determinations, eliminates 5-HIAA by extraction into ethyl acetate.

MATERIALS AND METHODS

Reagents. Butyl acetate (chromat. grade) is washed successively with N NaOH, N HCl and twice with distilled water. Phosphate buffer: 0·1 M (KH_2PO_4 + Na_2HPO_4) pH 6·5, to which 0·1% ascorbic acid is added just before use. Borate buffer: 0·5 M (H_3BO_3 + NaOH) pH 10. Butanol is washed successively with (2 N NaOH, distilled water, 2 N HCl, distilled water), twice, distilled water, and then saturated with borate buffer and NaCl. Heptane is washed successively with (2 N NaOH, distilled water, 2 N HCl, distilled water), twice. Ninhydrine solution: 0·1 M ninhydrine in 0·1 ml phosphate buffer pH 6·5.

Brains from male Sprague–Dawley rats, weighing 200–220 g, are homogenized in 0·4 N perchloric acid in a Potter–Elvehjem homogenizer. To 1 g of brain tissue in 20 ml of homogenate, 50 mg of EDTA and 50 mg of ascorbic acid are added and thoroughly mixed. The homogenate is centrifuged at 4500 rpm for 5 min and 10 ml (0·5 g) of the clear supernatant are used for the determination of 5-HT and 5-HIAA. The pH of the supernatant is adjusted to 6·8–7·0 by means of solid K_2CO_3. 1 ml of a 10% $ZnSO_4$ solution and 0·3 ml of 1 M NaOH are added and thoroughly mixed. After 5 min centrifugation 3 g of solid NaCl, 0·3 ml of 6 N HCl and 10 ml of butyl acetate are added. The tube is shaken for 5 min and then centrifuged for 5 min at 4500 rpm.

For the determination of 5-HIAA 9·5 ml of the clear organic layer are transferred to another centrifuge tube containing 1·5 ml of 0·1 M phosphate buffer, shaken, and centrifuged for 5 min. 0·5 ml of HCl conc. is then added to 1 ml of the buffer phase and the fluorescence measured in an Aminco–Bowman spectrofluorometer at activation and fluorescence wavelengths of 305 mμ and 540 mμ respectively (Multipl. 0·001; Sensit. 50; Slits 5-2-3; 3-2-5; Photomultipl. 4).

For the determination of 5-HT the aqueous phase is brought above pH 10 by means of solid K_2CO_3 and 2 ml 0·5 M borate buffer pH 10, 25 ml N butanol and 3 g NaCl are added. The tube is shaken and centrifuged for 5 min and 20 ml of the organic layer is transferred to another centrifuge tube containing 30 ml N heptane and 2·5 ml 0·1 M phosphate buffer pH 6·5. After shaking and centrifugation at 4500 rpm for 5 min, 1 ml of the buffer layer is mixed with 0·2 ml of ninhydrine solution and incubated at 75° for 30 min. The formed fluorescence is measured 1 hr after the end of the incubation in an Aminco–Bowman spectrofluorometer at activation and fluorescence wavelengths of 385 mμ and 490 mμ respectively (Multipl. 0·01; Sensit. 50; Slits 3-2-3; 3-2-3; Photomultipl. 4).

0·4 N $HClO_4$ blanks are treated as described above. Calculations are made with reference to the value of the internal standard which is carried through the entire estimation procedure.

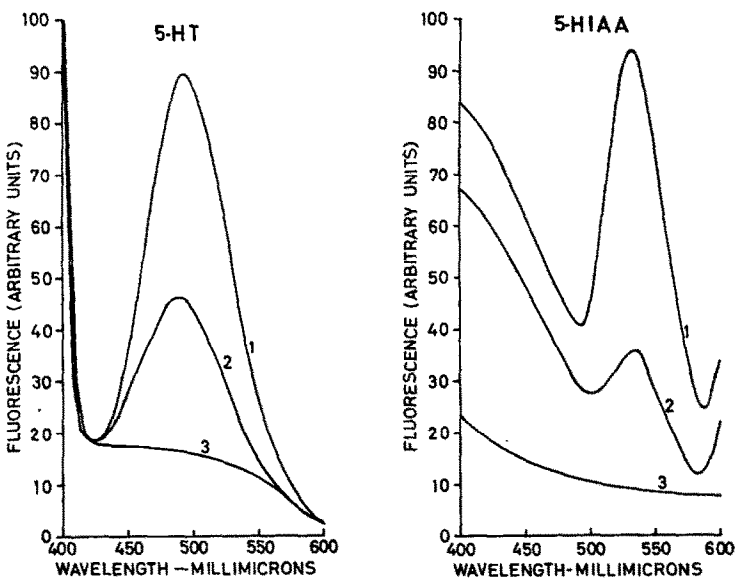

FIG. 1. (a): 1: Fluorescence spectrum of 5-HT internal standard (0·5 μg 5-HT added to 500 mg of brain tissue).
2: Fluorescence spectrum of 5-HT in 500 mg of brain tissue.
3: Blank.
(b): 1: Fluorescence spectrum of 5-HIAA internal standard (0·5 μg 5-HIAA added to 500 mg of brain tissue).
2: Fluorescence spectrum of 5-HIAA in 500 mg of brain tissue.
3: Blank.

The fluorescence spectra of 5-HT and 5-HIAA, expressed in arbitrary units of fluorescence, are given in Fig. 1. Rat brain 5-HT and 5-HIAA amounted to 0·600 ± 0·032 μg/g and 0·361 ± 0·015 μg/g, respectively. The results obtained are in agreement with the values of 5-HT found previously by two[8] of us in a large number of animals and with the 5-HIAA values reported in the literature.[6-9]

No reciprocal influence of 5-HT and 5-HIAA were observed in the simultaneous estimations performed in this study. As can be seen from the S.E.'s reported, the reproducibility of the method described is very satisfactory.

In conclusion, the present method is simple and reliable. It offers the possiblity to study 5-HT/5-HIAA ratios in the same sample, and, accordingly, changes in the metabolism of 5-HT under the influence of pharmacological substances.

J. F. and C. Heymans Institute of Pharmacology,
University of Ghent, Belgium

U. SCAPAGNINI*
R. VANDENBROECK
A. DE SCHAEPDRYVER

* Italo–Belgian Exchange Research Fellow from the 2nd Chair of Pharmacology, University of Naples, Italy.

Present address: Department of Physiology, University of California Medical School, San Francisco, California, U.S.A.

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The influence of oxotremorine on iron and flavines in mitochondria from rat brain corpus striatum*

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THERE is much evidence to support the importance of corpus striatum in the genesis of static tremor.[1,2] The area of globus pallidus seems to be particularly involved in the development of tremor, since its destruction in Parkinsonians leads to a marked alleviation of symptoms.[3] Globus pallidus is an area of considerable biochemical interest for its high concentration of iron[4] and flavines.[5] Therefore a knowledge of the behavior of these factors during tremor is desirable. We have been able to show in earlier reports that tremor-producing drugs, such as tremorine (1:4-dipyrrolidinobutyne), and oxotremorine (2′-oxo-1:4-dipyrrolidinobutyne), cause a considerable decrease of iron[6] and flavines in whole rat brains[7] and rat corpora striata.[8] The present work was intended to study parallel changes of iron and flavine concentrations under the influence of oxotremorine, and, in view of a possible metabolic meaning of such changes, rat corpus striatum mitochondria were examined instead of whole tissue.

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