Accumulation of 5-hydroxytryptophan in mouse brain after decarboxylase inhibition

The amount of 5-hydroxytryptophan (5-HTP) normally present in the brain is too small to be detected by any of the methods so far available. Wiegand & Scherfling (1962) have reported a content of 5-HTP in mouse brain of less than 0·1 μg/g and Lindqvist (unpublished) has found a value of less than 0·07 μg/g rat brain and less than 0·03 μg/g mouse brain. We have now investigated the accumulation of 5-HTP in mouse brain after decarboxylase inhibition.

Groups of 13–14 white female mice (NMRI), 18–24 g, were injected with a single dose of the aromatic amino-acid decarboxylase inhibitor Ro 4-4602[N^1-(DL-seryl)-N^2-(2,3,4-trihydroxybenzyl)hydrazine], 800 mg/kg intraperitoneally. The animals were decapitated at time intervals after the injection, the brains quickly dissected and each brain immediately homogenized with ice-cold perchloric acid containing ascorbic acid and EDTA (disodium ethylenediamine tetra-acetate). The interval between killing an animal and homogenization of the brain was less than 15 s. 5-HTP was isolated on a Dowex 50, X-4 column according to an unpublished method of Lindqvist.

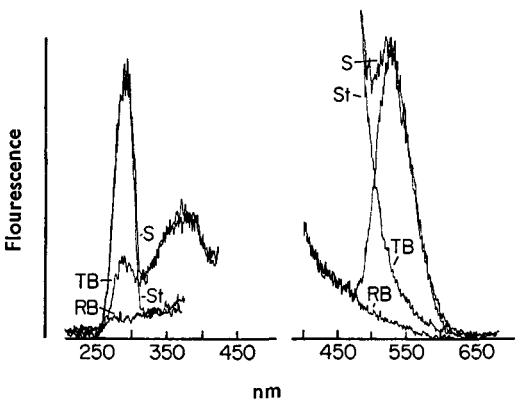

FIG. 1. Activation (left) and fluorescence (right) spectra of mouse brain samples. Ro 4-4602, 800 mg/kg i.p., was given 2 h before killing the animals. The activation spectrum was recorded at a fluorescence wavelength of 545 nm. The fluorescence wavelength was recorded at an activating wavelength of 285 nm. An ultraviolet filter was placed in front of the photocell for elimination of the second order light scatter peak at 570 nm. St = 0·1 μg authentic 5-HTP per 1·6 ml. S = sample. TB = tissue blank. RB = reagent blank.

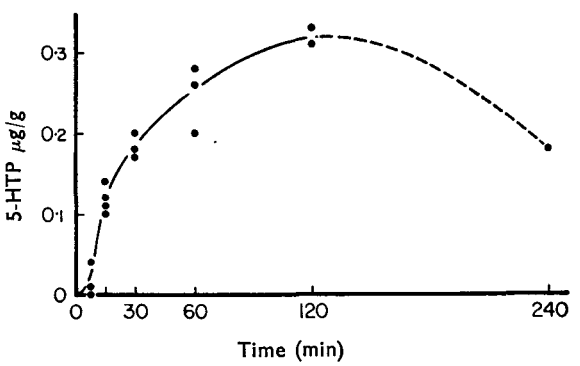

FIG. 2. Accumulation of 5-HTP in mouse brains at various time intervals after injection of Ro 4-4602, 800 mg/kg i.p. Each dot represents one determination on 13–14 pooled brains.

The fluorimetric assay was as for 5-hydroxytryptamine (5-HT) (Andén & Magnusson, 1967). Fig. 1 shows typical spectra of 5-HTP in brain samples of mice treated with Ro 4-4602, 4 h before death.

The concentrations of 5-HTP in mouse brains at various times after Ro 4-4602, 800 mg/kg, are shown in Fig. 2. A rapid accumulation of 5-HTP was seen between 7·5 and 15 min after the injection of the decarboxylase inhibitor. The highest amounts were found after 2 h, about 0·3 μg/g brain. After 4 h the values had declined, probably because of the short duration of action of the inhibitor. The accumulation of 5-HTP between 7·5 and 15 min (about 0·1 μg/g) corresponds to a synthesis rate of 5-HT of 0·8 $\mu g/g\ h^{-1}$. Other authors have reported a turnover rate of 5-HT in rat brain of 0·3 $\mu g/g\ h^{-1}$ (Diaz, Ngai & Costa, 1968). Whether this difference represents a real discrepancy, remains to be elucidated.

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The quantitative analysis of alkyl polyoxyethylene glycol monoethers with mass spectrometry and proton magnetic resonance spectroscopy in combination

The most recent spectroscopic method to be applied to the analysis of non-ionic surfactants is proton magnetic resonance (pmr) spectroscopy which, apart from qualitative data, has the added advantage of providing quantitative data without the use of standard compounds, provided a suitable internal standard, like the aromatic protons of a polyoxyethylene alkylphenol, is present in the molecule.

We now report the use of a combination of pmr spectroscopy and mass spectrometry in the examination of the purity of samples of dodecyl tetra-, hexa- and octa- oxyethylene glycol monoethers ($C_{12}E_4$, $C_{12}E_6$ and $C_{12}E_8$, respectively) which were prepared (Corkill, Goodman & Ottewill, 1961) for experiments reported elsewhere (McDonald, 1969). The mass spectra of these samples showed, as expected, molecular ions at m/e = 362, 450 and 538, respectively, and the expected fragmentations by stepwise loss of CH_2 and ($O{\cdot}CH_2{\cdot}CH_2$) units, indicated by a series of peaks at $M^+ -14$, $M^+ -28$, $M^+ -42$, etc. and $M^+ -44$, $M^+ -88$, etc., respectively, in each spectrum. Small peaks at m/e = 376 ($M^+ +14$) (13% of the molecular ion peak) and at m/e = 390 ($M^+ +28$) (5% of the molecular ion peak) were present in the spectrum of $C_{12}E_4$, indicating the presence of homologous impurities ($C_{12}E_4 + CH_2$) and ($C_{12}E_4 + 2CH_2$), respectively, in the sample. Similarly, small peaks at m/e = 464 ($M^+ + 14$) (1% of the molecular ion peak) and at m/e = 478 ($M^+ +28$) (5% of the molecular peak) were present in the spectrum of $C_{12}E_6$, indicating the presence of impurities of molecular formula ($C_{12}E_6 + CH_2$) and ($C_{12}E_6 + 2CH_2$), respectively, whereas the spectrum of $C_{12}E_8$ had no peaks of