Fluorometric determination of 3-methoxytyramine and 3-methoxy-4-hydroxyphenylethanol

The implication of cerebral dopamine in the pathophysiology of Parkinson's disease has stimulated further interest in the metabolism of dopamine and in developing methods for its quantitative determination and that of its metabolites. 3-Methoxy-tyramine (3-MT) is formed by *O*-methylation of dopamine through the action of catechol-*O*-methyl transferase (COMT). The intermediate aldehyde derived from oxidative deamination of 3-MT is reduced to the alcohol, 3-methoxy-4-hydroxy-phenylethanol (MOPET). Similarly, the action of monoamine oxidase on dopamine yields an intermediate aldehyde which can be reduced by aldehyde reducatase and subsequent *O*-methylation by COMT to form MOPET. The oxidation of the aldehyde intermediates represents the major pathway for dopamine metabolism in extracerberal tissue while MOPET is reportedly the major metabolite of exogenous dopamine in the brain (Taylor & Laverty, 1969). The presence of endogeneous amounts of MOPET in the rat brain have been recently demonstrated (Karoum, Ruthven & Sandler, 1971; Braestrup, 1972).

Methods for the determination of MOPET by gas chromatography have recently become available (Karoum & others, 1971; Wilk, 1971; Braestrup, 1972) and the fluorometric assay of 3-MT (Kakimoto & Armstrong, 1962; Carlsson & Waldeck, 1964) has been modified (Laverty & Taylor, 1968; Käser, 1970; Käser & Thomke, 1970; Guldberg, Sharman & Tegerdine, 1971). We have developed a simple and sensitive fluorometric assay for the estimation of MOPET and 3-MT.

The following optimal experimental conditions were determined for the quantita-tive fluorometric estimation of authentic MOPET and 3-MT: To 0·5 ml of sample con-taining 3-MT or MOPET, add 0·25 ml of 1·0 M phosphate buffer pH 8·5 (secondary and tertiary potassium salts). After the pH of the mixture is adjusted to pH 8·5, 2·2 ml of freshly prepared 1·0 M phosphate buffer saturated with NaCl, pH 8·5, is added to the sample mixture. The oxidation is effected by the addition of 0·05 ml of 10 mM sodium periodate solution. The reaction mixture is shaken briefly (Vortex) and allowed to stand for 8–10 min at room temperature (25°). Thereafter, 0·05 ml of freshly prepared solution of 2·5 % anhydrous sodium sulphite (w/v) in 5 N sodium hydroxide is added. The fluorophor formed is read after 15 min, in a spectrofluorometer at an emission wavelength of 440 nm when the sample is activated at 322 nm.

To test the specificity of the method, several structurally similar compounds were separately assayed (at a concentration of 2 μg per assay mixture) to determine their possible interference in the fluorometric estimation of 3-MT and MOPET. These compounds were 2,4-dihydroxyphenylalanine (DOPA), dopamine, adrenaline, noradrenaline, metanephrine, normetanephrine, 3,4-dihydroxyphenlacetic acid, 3,4 dihydroxymandelic acid, vanillylmandelic acid, homovanillic acid, tyramine 3,4-dimethoxyphenethylamine, 3-methoxy-4-hydroxyphenylglycol, 5-hydroxytryptophan-5-hydroxytryptamine and 5-hydroxyindoleacetic acid. With the exception of homo-vanillic acid, these compounds contributed a fluorescence less than 2·0 % of that of an equal amount of MOPET or 3-MT. 3-MT and MOPET could be determined in amounts as low as 15 and 20 ng ml^{-1} of the reaction mixture respectively. Linear relations between the concentrations of 3-MT and MOPET and fluorescence intensity are observed up to 6 μg ml^{-1} of assay mixture. The fluorophor formed is stable at room temperature (25°) for at least 2 h.

Oxidation of 3-MT at pH 6·5 with iodine according to the trihydroxyindole assay of Carlsson & Waldeck (1958) gave less fluorescence than did oxidation with periodate with maximal exitations and emissions occurring at 330 and 380 nm, respectively, This is in agreement with the finding of Anton & Sayre (1964), in which the oxidation

of dopa and dopamine with periodate yielded greater fluorescence than did iodine oxidation. However, our use of periodate oxidation at an alkaline pH followed by tautomerization in alkaline sulphite solution without a subsequent acidification (Carlsson & Waldeck, 1958) or heating in water bath, resulted in a shift of emission spectra to wavelengths of 440 nm when the reaction product was activated at 330 nm. This is advantageous since at this pH both dopa or catecholamines, if present, will be destroyed and thereby produce minimal interference in the fluorometric assay.

Due to similarities in the reactions employed for the estimation of 3-MT and MOPET, it is necessary to separate the two compounds before their quantitative determination. This can be accomplished by chromatography of a solution of the compounds on the cation exchange resin, Amberlite CG-50, at pH 6·5. MOPET passes through the column and can be extracted with the organic solvents recommended for the removal of phenylethanol and phenylglycol compounds (Goodall & Alton, 1968). 3-MT can be eluted by 2 N acetic acid.

The assay has been in use in our Laboratory for the determination of urinary 3-MT utilizing the isolation procedure of Bigelow & Weil-Malherbe (1968), by which both metanephrine, normetanephrine, and 3-MT are present in the same acid eluates of the Amberlite CG-50 column. The quantitation of low concentrations of 3-MT and MOPET should make this procedure applicable to tissue extracts.

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