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Note

New high-performance liquid chromatographic procedure for the detection and quantification of β-phenylethylamine

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β-Phenylethylamine (PEA) (1a, see Fig. 1) is an endogenous amine present in several mammalian tissues including the brain [1]. PEA elicits pharmacological actions either directly [2, 3] or indirectly through catecholaminergic [4] mechanisms.

PEA is unique among endogenous amines in that when applied exogenously this amine elicits behavioral stimulation in animals similar to the effects induced by amphetamine [5]. Its mechanism of action is not fully understood. However, it is possible that PEA interacts with specific receptors [3]. Indeed, the proposal has been made that PEA is an endogenous stimulant which is involved in the neurohumoral regulation of mood and activity [6]. Levels of endogenous PEA and of one of its metabolites, phenylacetic acid, may be altered in patients suffering from schizophrenia [7] or depression [8]. Thus, the availability of simple and sensitive procedures for quantifying levels of endogenous PEA clearly may have considerable practical significance. To date, PEA has been quantified reliably in biological fluids by means of gas chromatography–mass spectrometry [9–11]. These procedures, which are very accurate, involve the use of expensive equipment not routinely available in clinical laboratories. In this report we describe a very sensitive high-performance liquid chromatographic (HPLC) procedure

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using electrochemical detection (ED) for the determination of PEA levels. Owing to the lack of hydroxyl groups in the aromatic ring, PEA itself gives no signal in this ED system even at high oxidation potentials. The procedure presented here utilizes the chemical derivatization of PEA with an acyl group that is readily oxidizable in the electrochemical detector [12, 13]. With an optimal internal standard it is possible to achieve high sensitivity and precision. This strategy was previously used for the HPLC–ED analysis of histamine [14].

EXPERIMENTAL

Materials

β-Phenylethylamine hydrochloride was purchased from Sigma (St. Louis, MO, U.S.A.). *p*-Tolylethylamine (1b) was purchased from Aldrich (Milwaukee, WI, U.S.A.). Sulfosuccinimidyl-3-(4-hydroxyphenyl)propionate (sulfo Bolton–Hunter reagent; 2) was from Pierce (Rockford, IL, U.S.A.). For structures, see Fig. 1.

High-performance liquid chromatographic equipment

The system consisted of a Gilson Instruments HPLC Model 302 pump (Gilson International, Middleton, WI, U.S.A.), a Rheodyne injector (Model 7215, Rheodyne, Berkeley, CA, U.S.A.), an ESA Coulochem electrochemical detector (Model 5100A, ESA, Bedford, MA, U.S.A.) with an analytical cell containing two electrodes in series (Model 5010, ESA) and a chart recorder (LKB, Bromma, Sweden).

The mobile phase was 70 m*M* sodium acetate (pH 3.2 with glacial acetic acid)–methanol (52:48, v/v). The column was a Supelco C_8, 3 μm particle size, 20 cm $\times$ 0.5 cm I.D. (Supelco, Bellefonte, PA, U.S.A.). The flow-rate was maintained at 1.5 ml/min. The first electrode was set at 0.3 V and the second electrode was set at 0.55 V.

Synthesis of standards

Internal standard (N-[3-(4-hydroxyphenyl)propionyl]-2-p-tolylethylamine). *p*-Tolylethylamine (88 mg, 0.65 mmol) and the Bolton–Hunter reagent (171 mg, 0.65 mmol) were mixed in 8 ml of a 1:1 solution of methanol and sodium phosphate buffer (1 *M*, pH 8). After 30 min, 8 ml of water were added. A crystalline solid was collected, washed with water and dried in vacuo giving 164 mg of the title compound in 89% yield, m.p. 120–121°C, CHN analysis.

PEA standard (N-[3-(4-hydroxyphenyl)propionyl]-2-phenylethylamine). It was prepared from PEA·HCl and Bolton–Hunter reagent using the procedure described above. The product was isolated in 75% yield, m.p. 102–104°C, CHN analysis.

Derivatization for HPLC analysis

Standards (1 ml) of PEA ranging from 0.6 to 10 ng/ml or 1 ml of 5 ng/ml *p*-tolylethylamine or water were mixed with 0.15 ml of a buffer consisting of 0.1 *M* Na_2CO_3–0.01 *M* $NaHCO_3$. The sulfo Bolton–Hunter reagent (100 μl, 700 μg/ml

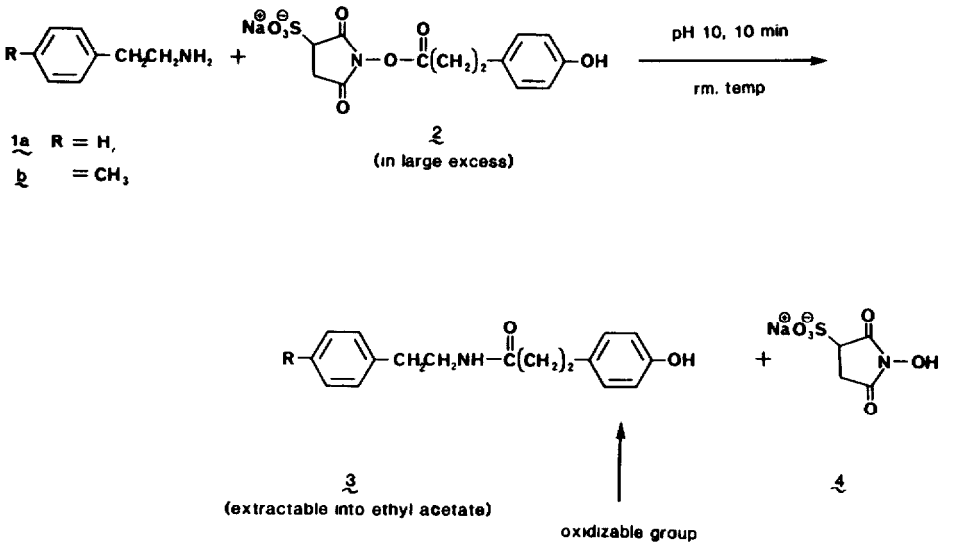

Fig. 1. Derivatization of PEA (1a) and the internal standard (1b) with the sulfo Bolton-Hunter reagent (2).

in distilled water) was added and the tubes were vortexed for 10 s. Ethyl acetate (1 ml) was added and the tubes were vortexed for 30 s and centrifuged at 1000 *g* for 10 min. The upper organic phase (800 μl) was transferred to another set of tubes and dried under nitrogen at room temperature. The residue was reconstituted with 500 μl of mobile phase and 20 μl were injected into the HPLC system.

RESULTS AND DISCUSSION

Reaction of PEA (1a) with an excess of sulfo Bolton–Hunter reagent (2) at pH 10 was essentially complete at room temperature in 10 min (Fig. 1). The product, 3, was extracted with ethyl acetate.

Standards of the Bolton–Hunter derivative of PEA gave a single symmetrical peak in the HPLC system. The retention time of 5–6 min is optimal for use in the analysis of PEA in biological material.

The detector response for a single concentration of PEA increased with the electrode voltage (Fig. 2), and 0.55 V gave the optimal signal-to-noise ratio.

The efficiency of the extraction procedure was tested with pure standards of the *p*-hydroxyphenylpropionyl derivative of PEA, 3a. The recovery was 95–100% under the present conditions and was not changed by altering the pH of the aqueous solution in the range 4–9, reflecting the high lipophilicity of the PEA derivative. When authentic PEA standards were derivatized under the described conditions, the peaks obtained showed the same retention time and height as the pure standards. Additional peaks appeared, however, in the chromatograms, representing probable breakdown products of the derivatizing reagent. Standard curves obtained were linear in the range tested, corresponding to the range of concentrations reported for biological levels [9–11]. A number of endogenous

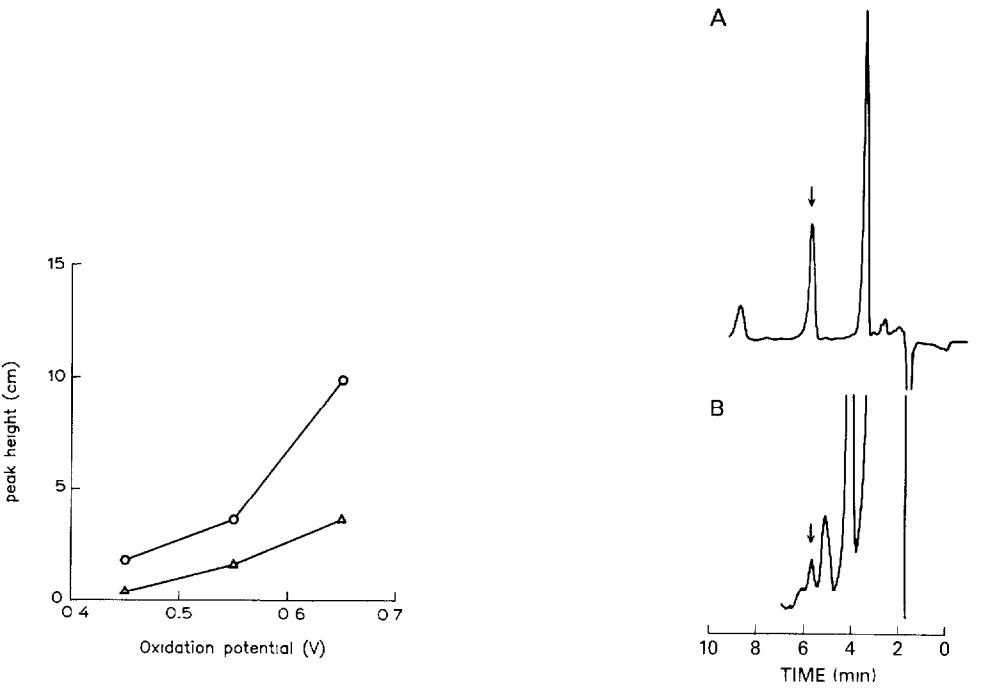

Fig. 2. Voltage–peak height response for the PEA (○) and the internal standard (△) Bolton–Hunter derivatives. A 10-ng amount of each derivative was injected at different voltage settings of the coulometric detector. HPLC conditions were as described in the text.

Fig. 3. Chromatograms showing (A) a standard of PEA Bolton–Hunter derivative (5 ng) and (B) a sample of primate cerebrospinal fluid (0.75 ng/ml) processed as described in the text. HPLC conditions were as described in the text. The arrows correspond to the PEA derivative peaks.

and non-endogenous, aliphatic and aromatic amines were derivatized and tested for possible interference in the chromatography. The amines tested when derivatized, did not interfere with the PEA Bolton–Hunter derivative peak (Table I). Ring mono- and dihydroxylated aromatic amines did not yield peaks in these chromatographic conditions. *p*-Tolylethylamine, 1b, was found to be a suitable

TABLE I

k' VALUES FOR DIFFERENT AMINES IN THE HPLC SYSTEM DESCRIBED

$k' = (t_{R_a} - t_{R_0})/t_{R_0}$ where t_{R_a} = retention time for the amine and t_{R_0} = retention time for solvent.

Amine	k'
Phenylethanolamine	1.3
Tryptamine	2.1
β-Phenylethylamine	2.8
Tranylcypromine	3.3
β-Tolylethylamine	4.7
Heptamine	4.9
Mephentermine	7.8

internal standard, e.g. of similar reactivity and polarity as PEA and giving an acylated derivative, 3b, with similar oxidation potential as compound 3a.

The procedure described here is of immediate practical use for the quantification of PEA in biological fluids. Preliminary data from our laboratory show that PEA can be detected and quantified readily in non-human primate cerebrospinal fluid (Fig. 3). An important advantage of this procedure is the fact that PEA can now be measured using inexpensive equipment. It should be noted that, since the completion of this work, an HPLC method for PEA has been reported which involves post-column derivatization and fluorometric detection [15].

The availability of this convenient HPLC–ED procedure for quantitative determination of PEA should lead to a greater understanding of the role of this amine in normal and pathological states. Finally, this procedure represents a further demonstration of the potential of sulfo Bolton–Hunter derivatization in the analysis of non-electroactive amines by HPLC–ED.

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