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Note

Amperometric determination of 3-methoxy-4-hydroxyphenylethyleneglycol in human cerebrospinal fluid

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3-Methoxy-4-hydroxyphenylethyleneglycol (MHPG) is the major central nervous system metabolite of norepinephrine [1—3] and measurement of MHPG levels in human cerebrospinal fluid (CSF) are an indication of central norepinephrine turnover. Methods which have been employed for the determination of MHPG in CSF include gas chromatography with electron-capture [4—7] or mass spectroscopic [8—12] (GC—MS) detection, fluorimetry [13, 14] and liquid chromatography (LC) [15, 16]. As methods of analysis have improved, reported mean values for free MHPG in normal or control human CSF have decreased from ca. 30 ng/ml [13, 14] and above [2, 7] to around 10 ng/ml [8, 10, 12, 17, 18]. Also, more recent reports state that the preponderance of the MHPG in human CSF is in the unconjugated (free) form [8, 10—12, 18]. In general, the procedures used have been lengthy and complicated. We present here a simple and rapid LC—amperometric method.

MATERIALS AND METHODS

MHPG standard was purchased from Sigma (St. Louis, MO, U.S.A.). A stock solution of 100 ng/ml in 0.1% (w/v) ascorbate was stable for up to three months.

The LC system was composed of an Altex 110A pump, a Rheodyne 71-20 injection valve with 100-μl sample loop (Rainen Instrument, Brighton, MA, U.S.A.), and a μBondapak C_{18} reversed-phase column (300 mm × 3.9 mm I.D., average particle size 10 μm, Waters Assoc., Milford, MA, U.S.A.). The ampero-

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metric detector consisted of a Bioanalytical Systems (West Lafayette, IN, U.S.A.) electrochemical controller (LC-2A), a glassy carbon working electrode, an Ag/AgCl reference electrode, and a Kel-F thin-layer detector cell and reference electrode compartment. A ca. 50-μm spacer gasket was used, and the working electrode was set at +0.75 V versus the reference electrode. The citrate—acetate buffer [19] solvent system was adjusted to pH 5.15 ± 0.01 and delivered at a flow-rate of 2.0 ml/min. The buffer was degassed by heating at ca. 45°C on a stirrer hot plate: the column apparently remained at room temperature as no change in retention time of MHPG standard was observed when the buffer was not warmed.

MHPG was determined in centrifuged (ca. 10,000 *g* for 2 min) lumbar CSF by injecting 20—50 μl of the otherwise unprocessed CSF. A 1-ng MHPG standard was injected after every two samples. A single-point standard (quantified by peak height) was used as the response was linear over the working range (0.1—1.0 ng). The full-scale sensitivity was usually set at 2 nA. Absolute background levels varied from ca. 0.5 to 3 nA, depending upon the particular electrode. The glassy carbon electrode was cleaned daily by wiping gently with a damp tissue.

RESULTS AND DISCUSSION

Chromatograms obtained from two different CSF samples are shown in Fig. 1; in each, 40 μl of centrifuged human lumbar CSF were injected directly. The difference in retention time observed for the MHPG peaks is due to the use of two different C_{18} μBondapak columns. The column employed for sample No. 1 had shown, even initially, lower retention times. The more extensive use of that column lowered retention times further and also lowered efficiencies to those observed. The MHPG peak was well formed and adequately separated from neighboring peaks using the pH 5.15 solvent system. In order to further establish the identity of the peak, CSF was also chromatographed using solvent systems of pH 3.75 and 6.00. Under these conditions, the peak still co-eluted with MHPG standard, having a lower retention time at lower pH and a greater retention at higher pH (retention time ratios at pH 3.75, 5.15, and 6.0, were 0.5, 1.0 and 1.1, respectively). At pH values other than ca. 5.15, the MHPG peak was not as well separated from nearby peaks. A similar study with 0.5% and 5% methanol added to the pH 5.15 buffer also confirmed the identity of the peak while giving inadequate separation. A very recent high-performance liquid chromatography—amperometric method [16] for determining MHPG in CSF apparently suffers from some degree of interference as a broadened peak was observed in samples.

A standard addition study with free MHPG (10, 20, and 40 ng/ml added) gave a linear recovery of 95.1 ± 5.9%. The compound was determined in a single CSF sample with a coefficient of variation of 7.3% (10.3 ± 0.75 ng/ml, mean ± S.D., n = 5). CSF samples examined to this point have had concentrations of 6.7—14.8 ng/ml, in agreement with GC—MS reports [8, 10, 12, 17, 18]. In order to speed up the determination, the late-eluting peaks can be rapidly removed by injecting about 1.0 ml of methanol. The detector cell was bypassed for 3—4 min while the methanol eluted. A steady baseline was obtained within 1 min of switching the detector back on-stream.

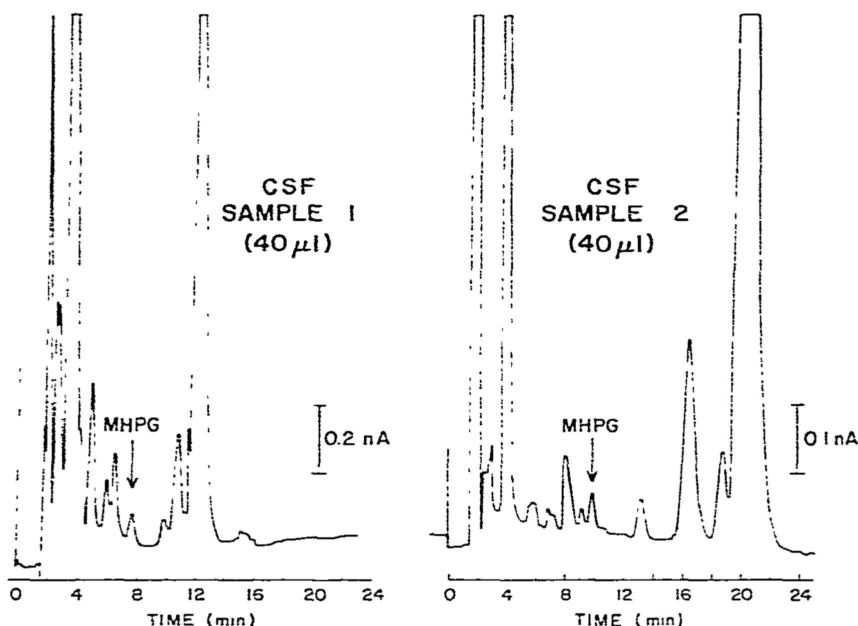

Fig. 1. The determination of MHPG in two different CSF samples using different C_{18} columns. The MHPG levels for the samples shown are 6.7 ng/μl (sample 1) and 10.5 ng/ml (sample 2). Electrode sensitivities established at the time each sample was run were 0.336 and 0.125 nA per ng MHPG injected for samples 1 and 2 respectively.

An attempt was made to extend the method to the determination of free MHPG in human urine and plasma. In urine, a peak co-eluted with MHPG standard using the pH 5.15 solvent system. However, when the peak was collected and re-chromatographed using a solvent system containing 5% methanol, the retention time was 4.5 min versus 4.3 min for MHPG standard (α = 1.07). In plasma, a poorly shaped, presumably merged, peak was observed, which when quantitated gave a much higher than expected concentration (ca. 50 ng/ml versus reported values of ca. 5—10 ng/ml).

We believe the method to be much simpler, less expensive, and more rapid than existing methods. Amperometric detection of LC eluents is not without its practical problems due to bubble formation, electrode passivation, and system shielding, etc. However, the advantages of the method are more than ample compensation for the occasional problem.

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