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Note

Determination of the amine metabolites of selegiline in biological fluids by capillary gas chromatography

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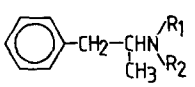
Selegiline (formerly known as L-deprenyl) is a monoamino-oxidase-B inhibitor, which decreases dopamine degradation at the neuronal level and is therefore used as an adjuvant to L-dopa treatment in Parkinsonism. Reynolds et al. [1] showed that biotransformation of this drug produces amphetamine and metamphetamine, which are excreted in the urine. In addition, desmethylselegiline is formed [2,3]. Each of the compounds (Fig. 1) has some biological activity. Determination of the metabolite concentrations after selegiline administration would provide information on their possible contribution to the observed drug effects.

Amphetamines have been of considerable interest in forensic science and toxicology, and an abundance of methods for their analysis exists. Few of these, however, allow for determinations at low ng/ml level. In addition, simultaneous assay of methamphetamine and amphetamine usually involves chromatographic separation. To quantitate still another amine metabolite makes further demands on the system. Juvancz et al. [4] succeeded in measuring the



SELEGILINE : $R_1 = CH_3$, $R_2 = CH_2C{\equiv}CH$
DESMETHYLSELEGILINE : $R_1 = H$, $R_2 = CH_2C{\equiv}CH$
METAMPHETAMINE : $R_1 = CH_3$, $R_2 = H$
AMPHETAMINE : $R_1 = H$, $R_2 = H$

Fig. 1. Structures of selegiline and its amine metabolites.

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parent drug, selegiline, but even their method has severe drawbacks: impractically large sample amounts (3–5 ml) are needed, and sample preparation by steam distillation is poorly compatible with serial analysis. More importantly, the assay does not quantitate the metabolites.

The objective of the present report is to show that amphetamine and the secondary amine metabolites of selegiline can be quantitated in a single gas chromatographic (GC) run as their trichloroacetamides. Enhanced electron-capture sensitivity of the derivatives, combined with effective separation in a fused-silica capillary column, allows for detection with high sensitivity.

EXPERIMENTAL

Chemicals

Pure selegiline hydrochloride and the internal standard, MPV-283 [2-(2,6-dimethylphenyl)ethylamine], were from Farmos Group (Turku and Oulu, Finland). Desmethylselegiline was obtained from Chinoin (Budapest, Hungary). Both D-amphetamine sulphate and D-metamphetamine hydrochloride were purchased from Sigma (St. Louis, MO, U.S.A.). 1,1,1-Trichloroacetyl chloride (TCAC) was from Ega-Chemie (Steinheim/Albuch, F.R.G.). Other chemicals were of analytical grade.

Extraction

To a 1-ml serum or (diluted) urine sample were added 0.5 ml of distilled water containing 400 ng of the internal standard and 50 μl of 5 *M* NaOH. The sample was extracted with 5 ml of *n*-hexane for 15 min in a reciprocating shaker. After phase separation by centrifugation, a 4-ml aliquot of the hexane extract was transferred to a clean, dry test-tube, and 10 μl of 1% (v/v) TCAC in hexane were added. The tube was closed with a screw-cap and incubated at 40 °C for 30 min. Then the organic phase was washed successively with 2 ml of 0.1 *M* NaOH and 2 ml of distilled water. Finally the hexane phase was evaporated to dryness in a stream of dry compressed air at 50 °C. The residue was dissolved in 200 μl of toluene and allowed to stand for at least an hour before a 2–3 μl sample was injected into the gas chromatograph.

In the standard series 1–1000 ng of each standard substance were included in the 0.5 ml of water added to blank serum. The rest of the extraction procedure was as above.

Chromatography

A Hewlett-Packard 5880 A gas chromatograph equipped with a ^{63}Ni electron-capture detector was used. The column was an Oribond® (Orion Analytica, Espoo, Finland) fused-silica capillary (12.5 m × 0.22 mm I.D.) with a cross-linked SE-30 coating. Injections were performed in the splitless mode, with a splitless time of 0.5 min. The injector temperature was 285 °C, and the detector

temperature 300 °C. The oven temperature was initially 90 °C for 0.6 min, then programmed at 30 °C/min to 155 °C, from 155 °C to 195 °C at 15 °C/min, and finally from 195 °C to 220 °C at 30 °C/min; it was then held isothermal until the end of the run (12.5 min). The inlet pressure of the helium carrier gas was 110 kPa, giving a linear velocity of 40 cm/s at the starting temperature. The flow-rate of the make-up gas (argon–methane) was 35 ml/min. Peak-height ratios were used for quantitation.

Animal application

Four beagle dogs were dosed orally with 3 mg/kg and two weeks later with 10 mg/kg selegiline hydrochloride, weighed in gelatine capsules. The dogs were kept in metabolic cages and their daily urine was collected for three days. Blood was sampled at preselected times for three days also. Sera were separated. Samples were stored deep frozen at −20 °C until analysis.

RESULTS AND DISCUSSION

Derivatization

Pentafluorobenzoyl (PFB) derivatives of amphetamine and related primary amines have generally given the best electron-capture sensitivity [5–8]. With the exception of Terada et al. [7], authors have reported much lower responses for the secondary amine PFB derivatives. In this study derivatization with PFBCl was also tested under the conditions described by Terada et al. [7]. The resulting peak height of PFB-metamphetamine was only ca. 30% of the PFB-amphetamine peak height. In addition, a somewhat noisy chromatographic baseline was obtained. Derivatization of desmethylselegiline was not tested.

Trichloroacetyl derivatives of amines have been reported to show an electron-capture response nearly as high as that of pentafluorobenzamides [5]. Good chromatographic behaviour is also one of the features described for trichloroacetamides [5,9]. The present study confirmed these findings. The TCA amides of amphetamine and metamphetamine gave strong, essentially similar electron-capture responses. A pronounced signal was also obtained from the TCA derivative of the internal standard (a primary amine). Desmethylselegiline reacted reproducibly but, probably owing to the propargyl group in close proximity to the trichloroacetyl moiety, produced a weaker signal. Its response was about one-third of that of the other amines. As a tertiary amine, selegiline itself did not react under the conditions used here.

Chromatographic separation

With derivatization a major portion of the nitrogen atom's basicity in these amines is abolished. Consequently, adsorption onto the column diminished and peak shapes are improved. Another contributory factor is the solvent effect brought about by splitless injection of samples in toluene at 90 °C. Polarity

differences of the derivatives are smaller than those of the parent amines. Separation of amphetamine and metamphetamine from each other and from desmethylselegiline was readily achieved on OV-1 as well as on SE-30. The best separation from background interferences was obtained on SE-30. Combined use of this column with trichloroacetylation resulted in relatively clean chromatograms (Figs. 2 and 3). However, baseline separation of desmethylselegiline and the internal standard could not be achieved because of the compromise between separation and quantitation efficiency for all the three amines. Consequently quantitation of desmethylselegiline concentrations below 5 ng/ml was inaccurate. When necessary, a small improvement in the separation of these two compounds can be gained by altering the temperature programme.

Fig. 2. Chromatograms of dog plasma: (left) drug-free sample with added internal standard, 200 ng/ml, retention time = 10.13 min; (right) dog plasma 10 min after p.o. administration of 10 mg/kg selegiline hydrochloride. The peak at 7.02 min corresponds to 13.3 ng/ml amphetamine, the peak at 8.67 min to 60.3 ng/ml metamphetamine, and the peak at 9.93 min to 37.1 ng/ml desmethylselegiline. The small serum peak at 8.73 min is baseline-separated at low methamphetamine concentrations. At higher concentrations it merges with the tail of the metamphetamine peak but does not significantly affect its height.

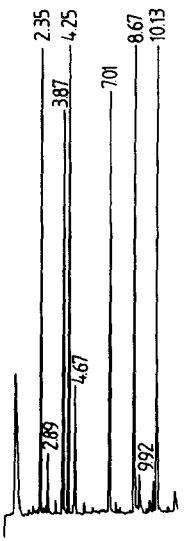

Fig. 3. Chromatogram of dog urine collected 0–7 h after p.o. administration of 10 mg/kg selegiline hydrochloride (100 μl of urine was diluted with 900 μl of water before extraction). The peak at 7.01 min corresponds to 47 ng/ml amphetamine, the peak at 8.67 min to 115 ng/ml methamphetamine, and the peak at 9.92 min to 14 ng/ml desmethylselegiline.

Quantitation

The smallest detectable level with a signal-to-noise ratio of 3 was ca. 1 ng/ml for amphetamine and metamphetamine, and ca. 3 ng/ml for desmethylselegiline. Thus the detection limit for amphetamine was approximately what has been reported with packed-column GC and derivatization with TCAC [9] or PFBCl [8]. Terada et al. [7] were able to detect metamphetamine in urine down to 10 ng/ml. With the the present assay system similar metamphetamine concentrations in urine could be analysed. The detection sensitivity of desmethylselegiline was not given in the papers reporting it as a metabolite [2,3], which precludes any comparison.

Reproducibilities of ten parallel determinations were 7.1%, 6.2% and 6.8% at 100 ng/ml, 6.4%, 4.8% and 10.1% at 20 ng/ml, and 12.4%, 24.1% and 24.4% at 5 ng/ml for amphetamine, metamphetamine and desmethylselegiline, respectively. The deviation from the target value, 20 ng/ml, of a control sample in four consecutive series (accuracy) of amphetamine analysis was −4.8%, that of methamphetamine 0.4% and of desmethylselegiline −21.3%. Extraction efficiencies for all amines exceeded 80%. Linearity in the range 0–100 ng/ml was good. Correlation coefficients of the six-point standard curves (r^2, linear regression) were 0.9948 for amphetamine, 0.9971 for metamphetamine and 0.9984 for desmethylselegiline.

Metabolite kinetics in the dog

Following the 3 mg/kg dose, amphetamine and metamphetamine were measurable in serum until 48 h after administration, but desmethylselegiline could be detected only in 20, 40 and 80 min samples. At the higher dose amphetamines were detected until 48 h and desmethylselegiline until 4 h. Maximum concentrations of amphetamine were 102 ng/ml (at 2 h) and 190 ng/ml (at 4 h), those of methamphetamine 87 ng/ml (at 40 min) and 336 ng/ml (at 80 min) and of desmethylselegiline 11 ng/ml (at 40 min) and 64 ng/ml (at 20 min) for the 3 mg/kg and 10 mg/kg dose, respectively. The corresponding estimates of elimination half-lives for amphetamine were 7.6 and 6.0 h and for methamphetamine 5.7 and 2.7 h. For the elimination half-life of desmethylselegiline an estimate, 0.9 h, was obtained only with the higher dose. The following fractions of the dose were found in urine: amphetamine, 10.1% and 8.1%; methamphetamine, 2.4% and 4.5%; desmethylselegiline, 0.03% and 0.01%; and total 12.5% and 12.6% (at 3 mg/kg and 10 mg/kg, respectively).

Attempts to measure the parent drug, without derivatization, were unsuccessful because concentrations in serum were below the detection limit of the nitrogen–phosphorus detector.

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