The direct injection of deproteinized bound fraction by ultrafiltration termination of the non-protein-analysis-HPLC to determine assay. offer simplified alternatives for rapid

indicates that "N₂ (nitrogen molecule)" was used for the calculation. He apologizes for the confusion.

Total and Non-Protein-bound Fractions of 3,4-Dihydroxyphenylalanine

To the Editor:
Several analytical methods for the determination of plasma 3,4-dihydroxyphenylalanine (L-DOPA) by HPLC have been reported. Many of these methods require preliminary purification of the plasma sample. Such purification procedures include adsorption on alumina (1) and solid-phase (2) or solvent extraction (3). The direct injection of deproteinized plasma supernatant (4) and the determination of the non-protein-bound fraction by ultrafiltration (5) offer simplified alternatives for rapid assay.

Dethy et al. (6), propose microdialysis-HPLC to determine L-DOPA and its metabolites (3,4-dihydroxyphenylacetic acid, dopamine, and homovanillic acid) in the plasma of patients with advanced Parkinson’s disease. We would like to make a number of comments concerning this attractive new method. First, it should be noted that microdialysis excludes large molecules (the authors do not specify the molecular weight excluded by the membrane) and thus separates protein-bound L-DOPA that is not dialyzed from unbound L-DOPA that passes through the membrane. Although L-DOPA binds to plasma proteins only to a minor extent, the unbound fraction is influenced by the total concentration in the sample. In fact, it increases from an average of ~70% at 100 μg/L L-DOPA to ~90% at 1000 μg/L (7). Although for therapeutic drug monitoring purposes unbound plasma concentrations are considered to more accurately reflect drug concentrations at the site of action, variability in protein binding implies that results in plasma dialysates and whole plasma may not be comparable.

Another important issue is the poor stability of L-DOPA, particularly when it is separated from its plasma matrix. Addition of antioxidants like ascorbic acid (5 mM) or sodium metabisulfite (5 mM) does not markedly improve the stability of an aqueous solution of L-DOPA, whose concentration, even in the presence of the above antioxidants, decreases by 60% after 3 hours at room temperature (8). Moreover, antioxidants may increase the background current of the electrochemical detector and may give rise to additional unidentified peaks (8). It would, therefore, be important to determine the stability of the drug in plasma and dialysates using the antioxidant solution proposed by the authors.

Finally, with respect to the chromatographic separation, the peaks of L-DOPA, 3,4-dihydroxyphenylacetic acid, and DA are not well separated from each other, from the solvent front, or from two unidentified peaks. For these reasons, it would be useful to know the procedures used to confirm the identity of peaks and to exclude possible coelution with unidentified substances.

References

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PSA Concentrations in Seminal Plasma

To the Editor:
Prostate specific antigen (PSA) is an important tumor marker for the detection and monitoring of prostate cancer. PSA is secreted by the prostatic epithelial cells into the lumen of the prostate duct during the formation of seminal plasma. PSA is a 30 kDa serine protease that cleaves biological substrates in seminal fluid, including semenogelin I, semenogelin II, and fibronectin, into small peptides, resulting in increased sperm motility (1–4). PSA has also been shown to cleave other biological substrates, including insulin-like growth factor-binding protein-3 and laminin, indicating the potential role of PSA
in the regulation of various biological functions (5, 6). The cellular expression of PSA is under androgen regulation, and the reduction of androgen function (e.g., by administration of finasteride) has been shown to reduce prostate tissue expression of PSA (7, 8). Nonprostatic sources of PSA have now been documented, including the milk of lactating women, amniotic fluid, and cerebrospinal fluid (9).

We report here the PSA concentrations in seminal plasma collected under tightly controlled conditions from 22 young, healthy men. Such information has not been well defined in previous reports because of variability in the collection and storage conditions of seminal plasma (10, 11).

Samples were obtained with informed consent from 22 normospermic volunteers in the Male Fertility Research Program of The Miami Project to Cure Paralysis at the University of Miami School of Medicine, Miami, Florida. All of the volunteers were in good health with no history of infertility or genitourinary disease. Their mean age was 30.3 ± 1.5 (SE) years (range 19–44 years). The subjects produced specimens by masturbation after at least 3 days of abstinence. Aliquots of the raw semen (0.5 mL each, stored in 1.5 mL Eppendorf tubes) were placed in a −80 °C freezer exactly 15 min after collection. The specimens were stored 2–12 weeks before PSA analysis.

The PSA determination was performed using the Hybritech Tandem®-MP PSA assay. Multiple dilutions (n = 4) of each specimen were analyzed, and concentrations were calculated by the multiplication of assay results of diluted samples by the dilution factors. The CVs for the mean concentration of the 4 results was <10% for 20 of the 22 determinations and <20% for all of the determinations.

The range of PSA concentration was 0.39–3 g/L (Fig. 1); the mean value was 1.29 g/L with a SD of 0.68 g/L. The median value was 1.17 g/L. The median value was 1.29 g/L with a SD of 0.68 g/L.

An appreciation of the wide range (10×) of seminal plasma PSA concentrations in healthy male donor specimens may be useful for studies of the biological roles of PSA and the potential effects of pharmaceutical agents on seminal plasma PSA concentrations.

Fig. 1. The distribution of seminal plasma PSA concentrations from 22 healthy donors.

References


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