donors, 3 of 10 male donors, and 6 of 11 patients with prostatic hyperplasia (on the basis of histology of transurethral resection tissue in 6 cases and clinical criteria in 5 cases). The average age (median, ranges) of the blood donors was 43 (19–82) years and of the men with hyperplasia was 67 (55–82) years. Cross-contamination, which may occur by nested PCR, is made unlikely by meticulous performance of the assay. Thus, negative controls were obtained via assay without added RNA or RT, respectively. SA, a restriction enzyme cutting the PSA target sequence but not that of hK2, digested the amplicons from two different-sized products. The PSA mRNA specificity of the RT-PCR product was further verified by sequencing the amplicons from these RT-PCR-positive control donors by the dideoxynucleotide chain termination method with a fluorescein-labeled primer on the automated DNA sequencer A.L.F. (Pharmacia, Freiburg, Germany). In all cases the sequence of the amplicon corresponded to the target PSA cDNA sequence published by Lundwall [9]. Therefore, the contamination by genomic DNA or hK2 is ruled out, too.

Recently, the expression of PSA in the mammary gland, endometrium, amniotic fluid, as well as in tumors of the mammary gland, liver, kidney, ovary, lung, and colon was identified by means of ultrasensitive immunossays. Diamandis points to the fact that, especially in cells responsive to steroid hormones, PSA might have functions that are not prostate-specific (see ref. 10).

With increases of sensitivity of the RT-PCR of PSA mRNA, the likelihood of getting a positive result with persons who do not have prostate cancer is increased. Further development of the detection of circulating prostatic tumor cells in blood requires the introduction of the quantitative RT-PCR. Recent data published byIsraeli et al. [8] and by Lorinc et al. [11] show that the efficacy of RT-PCR to detect circulating prostate cells on the basis of mRNA of the prostate-specific membrane antigen (PSM) is higher than that based on mRNA of PSA. Therefore, further development of a quantitative RT-PCR might show promise for targeting PSM mRNA.

This study was supported by a Charité research grant.

References


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Angiotensin Peptide Research Overlooked

To the Editor:

We disagree with the suggestion of Voelker et al. [1] that their report is the first application of the heptfluorobutyric acid (HFBA) solvent system for the separation and analysis of angiotensin peptides. In 1987, we published a HPLC/RIA method utilizing the HFBA solvent system that yielded superior resolution of angiotensin peptides [2]. In that report, we also discussed the enhanced resolution of HFBA, that this volatile solvent system would minimize interference with angiotensin RIAs, and that HFBA exhibits its greater selectivity for basic residues. Since that time, we have published numerous papers on the detection of angiotensin peptides, as well as the discovery of a novel angiotensin peptide [Ang-(1–7)], utilizing the HFBA-HPLC method coupled to RIA analysis. We have applied this method to assess angiotensin peptides in the plasma and tissues of several species [2–8]. Although Voelker et al. cited eight papers concerning angiotensin analysis, they did not cite any of our papers, despite their use of Ang-(1–7) as a calibrator in the separation of Ang II and related fragments. We have published >30 studies concerning Ang-(1–7) since 1988. We acknowledge that the Voelker paper is not an exact duplication of our own methodology. The separation conditions are not the same—isocratic vs gradient (Chappell), the concentration of HFBA and separation at 38 °C vs ambient temperature (Chappell). However, the Voelker et al. paper is on the separation and detection of the four Ang II-related peptides; our studies routinely measure 11 immunoreactive Ang peptides—Ang-(1–7), Ang II, Ang I, and their related peptide fragments. If the authors had attempted to measure these peptides, their conditions (i.e., gradient elution) might have paralleled our own.

References


3. Chappell MC, Brosnihan KB, Diz DI, Ferrario CM. Identification of angiotensin(1–7) in rat brain: evidence for differential processing of

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To the Editors:
We thank Chappell et al. for bringing to our attention their brief communication regarding HPLC separation of angiotensins [1]. We were unaware of this work. Reference to their HPLC method was not included in any of the method publications that we reviewed. Furthermore, three separate computer-based literature searches failed to identify the principal article describing their assay. One possible explanation was that we focused our literature review on HPLC-RiAs for Ang II in human plasma, and the references included in the letter by Chappell et al. are from investigations in nonhuman models, specifically dog, rat, and vascular smooth muscle cells. Recognizing the difficulty in familiarizing ourselves with all publications from the extensive literature pertaining to Ang II and related peptides, and the possibility that pertinent information may have escaped our review, we qualified the statement in our manuscript about the novelty of using a mobile phase containing HFBA to separate angiotensins.

Even though our chromatographic method [2] is similar to that described by Chappell et al. there are several important differences. Our HPLC conditions were optimized specifically for Ang II in human plasma, so that this peptide could be collected and analyzed in a single fraction. Because accurate quantification of Ang II requires reproducible elution and because peptide elution is highly sensitive to small changes in the amount of organic modifier, we developed an isocratic procedure with a washing and reequilibration step between injections. In contrast, the method by Chappell et al. involves gradient elution conditions in which the peptides are collected in multiple fractions. The collection and assay of multiple fractions is more labor intensive, time consuming, and costly. We did not demonstrate separation of Ang II from the same number of related peptides as Chappell et al. [3], because our goal was to resolve Ang II from peptides that cross-reacted with our anti-Ang II antibody. Thus, our investigation focused on separating Ang II from Ang 2-8 (Ang III), Ang 3-8, and Ang 4-8.

We regret that we failed to uncover and acknowledge the work by Chappell et al.; however, the results of both groups independently confirm the usefulness of HFBA as an ion-pair reagent for chromatographic separation of angiotensins. Future publications by our group concerning Ang II will include reference to the work by these investigators.

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Rare Interference in Determinations of Total Creatine Kinase

To the Editor:
Total creatine kinase (CK) from skeletal muscle may be a cause of error when released into the circulation (e.g., in cases of peripheral myopathy), where its activity contributes to that of total serum CK determined in patients with ischemic heart disease. However, and though extremely rare, total skeletal muscle CK activity may be added to total serum CK within the actual blood-collecting tubes if, after collection of the blood sample, muscle tissue is aspirated through the needle because of the negative pressure of the evacuated collection tubes used to extract the blood. This phenomenon occurred in the case we describe.

Our Emergency Laboratory was requested to determine the total CK and CK-MB in the evaluation of a patient tentatively diagnosed with acute myocardial infarction. The values of previous tests performed 6 h earlier were within the normal range for both analytes. The new assay (Tube 1) yielded CK of 2958 U/L (normal range, ≤200 U/L), and a CK-MB concentration of 36 U/L (normal range, ≤10 U/L), which represented 1.2% of the total CK. However, another tube of blood obtained at the same time (Tube 2) gave strictly normal values.

The results of the two tubes (in U/L) were as follows: Tube 1, CK 2958, lactate dehydrogenase (LD) 680, aspartate aminotransferase (AST) 147, CK-MB 36; Tube 2, CK 34, LD 427, AST 117, CK-MB 14. The rest of the biochemical analytes gave analogous results in both tubes. Samples collected later for assay of CK and CK-MB likewise yielded normal values.

Having excluded acute myocardial infarction in view of the clinical course and complementary explorations, and taking into account that only one tube exhibited abnormal results, we hemolyzed with distilled water all the blood in the sample tube that had given abnormal results (coagulation had been prevented with lithium heparin); a remaining particle was detected that histologically corresponded mostly to skeletal muscle, connective tissue, and fat. This suggested that the tissue had entered the tube through the needle, drawn in by the reduced pressure in the sampling tube.

Experimentally, we also found that a 7-mg fragment of muscle 1 mm in diam-