natriuretic peptides are stored in solutions (0.9% saline, phosphate-buffered saline, etc), their storage life is markedly less. When stored for 6 months at −80°C, the biologic activity as reflected by natriuresis or diuresis when infused into animals is no longer present in approximately 25% of the peptides thus stored. When this storage is continued for 8 months, 75% of the stored peptides no longer have biological activity. I hope this information will be useful to everyone with an interest in atrial natriuretic peptides.

David L. Vesely, MD, PhD
Professor of Medicine
University of South Florida
College of Medicine
Tampa, Fla

References

Stability of Plasma Atrial Natriuretic Peptide After Storage
To the Editor:
The stability of atrial natriuretic peptide (ANP) in plasma was reassessed on our part in response to the recent report by Nelesen et al,1 who claimed substantial loss of plasma ANP immunoreactivity after storage under most conditions. We would like to report our findings on the stabilization, storage, and assay of samples for ANP in support of Flynn et al,2 who have observed no significant degradation of ANP in stored samples.

We routinely have normal dog venous blood collected into chilled Vacutainer tubes containing EDTA, aprotinin, and sodium azide (at 1.5 mg, 1000 KIU [added immediately before collection], and 2 mg, respectively, per milliliter of blood) and centrifuged at 4°C; the resultant plasma is then frozen in dry-ice and stored at −80°C. For the present study, multiple portions of plasma were spiked with 0, 60, 150, 300, or 600 pg/mL (final concentrations) of ANP standard (human, synthetic; Sigma Chemical Co) and aliquoted into polypropylene tubes. Samples were taken either for immediate extraction on C-18 solid-phase columns (Waters) with subsequent radioimmunoassay (Peninsula antisera; NEN [125I]-rat ANP radiolabel) or quick-frozen and stored for 1, 5, or 8 weeks at −80°C before extraction and assay. Plasma from a single dog in experimental congestive heart failure (CHF) was collected in the same manner but not spiked with ANP standard and was included as a source of high levels of endogenous ANP.

There was no evidence that ANP in dog plasma stored frozen up to 8 weeks undergoes degradation. Control plasma consistently contained levels of ANP between 30 and 40 pg/mL. Observed values from spiked samples were generally 80% to 90% of expected values. Solid-phase extraction efficiency, as determined by recovery of radiolabeled ANP from dog plasma, was 80% to 85% and readily accounted for the lower values observed. Plasma ANP levels were consistent and high (~250 pg/mL) in the CHF dog, from immediate extraction until 8 weeks of storage at −80°C. ANP extracted from samples and refrozen at −80°C in assay buffer was also stable for at least 1 month.

In our hands, ANP does not undergo significant degradation in plasma when collected and stored with fidelity to the procedure above. The results of Nelesen et al could be attributed to the methods used in their treatment of the blood. For example, in their heparin and EDTA storage regimens, was blood stored frozen as stated by the authors? More important, they added the protease inhibitor aprotinin to the plasma, not whole blood, as we and others have done, such that substantial proteolytic degradation of ANP may have occurred by the time plasma samples were stored.

Magdi M. Asaad, PhD
Charles R. Dorso
W. Lynn Rogers
Bristol-Myers Squibb
Pharmaceutical Research Institute
Princeton, NJ

References

Reply
Vesely and colleagues draw their conclusions from their study1 in which they stored samples with less than 30 pg/mL ANP, an amount that provides less than 25% displacement of the radiolabeled ANP in their assay. We stored samples with 200 pg/mL ANP, so that if the samples degraded, ANP levels would remain in the sensitive range of the assay.2 Therefore, the lack of degradation found by Vesely may be due primarily to the low concentration of the samples.

Asaad et al claim no significant breakdown of ANP in dog plasma. Our study focused on human plasma samples. We made no claims as to these effects in other species. As to their questions of our methodology, the protease inhibitor was in blood collection tubes for that condition, and plasma, not whole blood, was frozen.

The concern expressed by these letters, as well as in the previous letters, is shared by us. Our findings are disconcerting for a dynamic field. The overall inconsistent findings that have been published, however, are just as disconcerting. As our study was done with three replications showing similar findings, we therefore stand by our findings that plasma ANP degrades during prolonged storage at −80°C.3 We recommend that plasma ANP samples not be stored for prolonged periods warmer than −196°C and not be subjected to more than a single thawing.

Richard A. Nelesen, PhD
Joel E. Dimsdale, MD
Michael G. Ziegler, MD
Departments of Psychiatry and Medicine
University of California, San Diego
La Jolla
Stability of plasma atrial natriuretic peptide after storage.
M M Asaad, C R Dorso and W L Rogers

Circulation. 1994;89:2457-2458
doi: 10.1161/01.CIR.89.5.2457

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