Disparity between studies of the stability of BNP in blood: comparison of endogenous and exogenous peptide

D R Murdoch, J Byrne, R Farmer, J J Morton

Measurement of plasma concentrations of the natriuretic peptides has recently been recognised as a potentially useful means of identifying patients with left ventricular systolic dysfunction (LVSD). Most studies, including our own, suggest that brain natriuretic peptide (BNP) may be superior to N-terminal atrial natriuretic peptide for diagnostic purposes. The widespread applicability of BNP would, nevertheless, be greatly diminished if the blood sample required special storage or handling. We have previously shown in a mixed population—incorporating patients with LVSD and healthy volunteers—that endogenous BNP is stable in whole blood at room temperature for three days. However, other groups have published conflicting results, which at first sight cast doubt on our data; therefore, our results have not gained universal acceptance. We, therefore, repeated our study and confirmed our original findings. Interestingly, our study is in agreement with the only other one to examine the stability of the endogenous peptide. This led us to believe that the disparity may have arisen because other groups have looked at the stability of exogenous rather than endogenous BNP. We report the results of a study directly comparing the stability of endogenous and exogenous BNP.

Methods

Blood was withdrawn from a forearm vein of 10 healthy volunteers (eight men, mean age 38 years) and divided directly into three chilled polypropylene tubes containing EDTA (1 mg/ml blood) and aprotinin (50 kIU/ml blood). The first sample was stored without the addition of exogenous BNP; 350 pg/ml of human BNP was added to the second sample; and the third sample was separated in a refrigerated centrifuge before the addition of the same concentration of BNP. We report the results of a study directly comparing the stability of endogenous and exogenous BNP.

Results

Endogenous plasma BNP concentrations in samples separated and frozen immediately ranged from 1.0 to 35.8 pg/ml (mean 14.8), and from 1.0 to 30.0 pg/ml (mean 11.7) following 72 hours’ storage at room temperature. The corresponding concentrations in spiked whole blood were 227.0 to 356.0 pg/ml (mean 298.5) immediately and 114.4 to 232.8 pg/ml (mean 188.6) after 72 hours’ storage; and for spiked plasma 303.0 to 396.0 pg/ml (mean 349.9) immediately, and 132.1 to 244.0 pg/ml (mean 195.4) following 72 hours’ storage (fig 1).

These data confirm our previous observations that only a minor decline in endogenous BNP concentration occurs over 72 hours at room temperature: mean change in concentration −18.5% (95% confidence intervals (CI) −8.0 to −28.9; p = 0.02 (paired two tailed t test)). A more pronounced decline in BNP concentration was noted in spiked samples over the same time period: whole blood −38.3% (95% CI −27.7 to −48.8; p < 0.0001); plasma −44.0% (95% CI −37.0 to −51.0; p < 0.0001). The decline in exogenous BNP was significantly greater than that for endogenous BNP whether stored as whole blood (p = 0.008, two sample t test % change) or plasma (p = 0.004).

Discussion

The decline in endogenous BNP reported here was slightly greater than we have previously found, but fewer subjects were studied, and the low endogenous BNP concentration from healthy volunteers were near the sensitivity.
limits of the assay. The much more dramatic
decline in exogenous BNP over 72 hours in
spiked blood and plasma samples, however,
remains unexplained. Possibilities include ad-
herence of the peptide to platelets, inherent
instability of the manufactured peptide, or
enzymatic degradation not inhibited by apro-
tinin. Interestingly, the 40% decline in BNP
concentrations that we observed in spiked
samples over 72 hours was still substantially
less than the 90% fall over 24 hours previously
reported.3

Our studies continue to support the feasibil-
ity of the assay of BNP for diagnosis of LVSD
in routine clinical practice.

1 McDonagh TA, Robb SD, Murdoch DR, et al. Biochemical
detection of left-ventricular systolic dysfunction. Lancet
2 Murdoch DR, Byrne J, Morton JJ, et al. Brain natriuretic
peptide is stable in whole blood and can be measured using
a simple rapid assay: implications for clinical practice.
3 Tsuji T, Imagawa K, Masuda H, et al. Stabilization of
human brain natriuretic peptide in blood samples. Clin
4 Dickstein K. Natriuretic peptides in detection of heart fail-
5 Prasad K, Fredericks S, Holt DW. Measuring brain
6 Penney MD, Hampton D. Assessment of heart failure
with plasma natriuretic peptides [letter]. Lancet 1998;351:
444.
7 Davidson NC, Coutie WJ, Struthers AD. N-terminal
proatrial natriuretic peptide and brain natriuretic peptide
are stable for up to 6 hours in whole blood in vitro. Circula-