Stabilization of Human Brain Natriuretic Peptide in Blood Samples

To the Editor:

Since the discovery of brain (or B-type) natriuretic peptide (BNP) of porcine origin, it has been suggested that the water-electrolyte balance is under the dual control of atrial natriuretic peptide (ANP) and BNP (1). The major origin of secretory human BNP (hBNP) is the ventricles of the heart, whereas human ANP (hANP) mostly originates from atria (2). The circulating hBNP is known to occur in at least two molecular forms, hBNP(77-108) (hBNP-32) and hBNP(1-108) (prohBNP) (3).

Plasma hBNP is markedly increased in patients with congestive heart failure (2, 4) and acute myocardial infarction (5), in proportion to the severity of the problem. To study the stability of hBNP in blood samples, we used a recently developed sensitive two-site immunoradiometric assay (IRMA) for hBNP (6). The system comprises two monoclonal antibodies, mAb1 and mAb2 (2), and recognizes the hBNP regions that maintain the integrity of both the C-terminal region and the disulfide bond-mediated ring structure. In brief, a mixture of standard hBNP (4-2000 ng/L, 100 μL) or sample (100 μL), 125I-labeled mAb1 (200 μL), and a bead bearing immobilized mAb2 is incubated at 4°C for 20 h. The buffer for the calibrators is 0.1 mol/L sodium phosphate (pH 6.5) containing 0.3 mol/L NaCl, 2 g/L bovine serum albumin, 1000 Kallikrein inhibitor units (KIU)/mL aprotinin, 1 mmol/L EDTA-Na2, 0.2 mmol/L cysteine, 20 g/L bovine gamma globulin, and 1 g/L NaN3; the same buffer, but containing 0.05 g/L mouse gamma globulin instead of the bovine gamma globulin, is used with the radiolabeled antibody. After removing the supernatant by aspiration, we wash the antibody bead twice with 2 mL of washing buffer: 0.01 mol/L sodium phosphate (pH 7.0) containing 0.15 mol/L NaCl, 0.2 mol/L Tween 20, and 1 g/L NaN3. The radioactivity bound to the bead is counted in a gamma counter. A plot of the radioactivity counts vs the concentrations of hBNP calibrator is used to estimate the concentrations of plasma hBNP. Using this IRMA, we could detect 0.2 pg/tube (2 ng/L (0.6 pmol/L)) of hBNP-32-like immunoreactivity. The basal value for plasma hBNP in 40 healthy subjects was 6.4 ± 3.8 ng/L (1.8 ± 1.1 pmol/L).

We first examined the stability of hBNP in whole blood collected from healthy volunteers (Fig. 1). The samples contained EDTA-Na2 (1.5 g/L) and added hBNP-32 (170 ng/L (49.1 pmol/L)) for easier estimation of hBNP degradation. As shown in Fig. 1A, hBNP was 75% destroyed even at time zero (without storage) in the absence of protease inhibitor. This is in striking contrast to hANP, whose degradation at time zero is virtually negligible under the same conditions (7). This early loss of hBNP was mostly prevented by the addition of aprotinin (500 KIU/mL), a serine protease inhibitor, which maintained the hBNP concentration at 60% and 10% of its initial value after 3 and 24 h of storage at 25°C, respectively (Fig. 1A), and at 70% and 30% after 3 and 24 h at 4°C, respectively (Fig. 1B). However, phosphoramidon (2, 20, or 200 nmol/L), a metalloendopeptidase inhibitor, had no effect (Fig. 1). Addition of benzamidine (10 or 100 nmol/L) in combination with aprotinin (500 KIU/mL) effectively stabilized hBNP, maintaining the hBNP concentration at 80~100%, 40-50%, and 20-40% of its initial value after 3, 24, and 48 h of storage at 25°C, respectively (not shown). The hBNP concentration in the samples was found to be independent of the pH and NaCl concentrations of the buffer after dialysis at 4°C (8).

Fig. 1. Stability of hBNP in whole-blood samples at 25°C (A) and 4°C (B).

Samples were collected from three apparently healthy male volunteers (ages 25~27 years) into 50-mL plastic syringes and quickly transferred to ice-chilled plastic tubes containing EDTA-Na2 (1.5 g/L) and hBNP-S2 (170 ng/L, 49.1 pmol/L). The tubes also contained aprotinin (500 KIU/mL), phosphoramidon (10.9 μg/L), and no additives (A) or aprotinin (500 KIU/mL), benzamidine (100 nmol/L) (B). The blood samples were stored at 4°C for 30 min and then kept at 25°C (A) or 4°C (B) for 0.5, 3, 24, or 48 h. After this, 1-mL aliquots were withdrawn and centrifuged at 1600g and 4°C for 20 min. The resulting plasma samples were kept frozen at -80°C until determination. hBNP-32-like immunoreactivity was determined with the IRMA described elsewhere (8). The data represent mean ± SD of three determinations.