Vol. 180, No. 1, 1991
October 15, 1991

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 431-436

Augmented Secretion of Brain Natriuretic Peptide in Acute Myocardial Infarction

Masashi Mukoyama[1], Kazuwa Nakao[1]*, Kenji Obata[2], Michihisa Jougasaki[2], Michihiro Yoshimura[2], Etsuo Morita[2], Kiminori Hosoda[1], Shin-ichi Suga[1], Yoshihiro Ogawa[1], Hirofumi Yasue[2], and Hiroo Imura[1]

[1]Second Division, Department of Medicine, Kyoto University School of Medicine, Kyoto 606, Japan

[2]Division of Cardiology, Kumamoto University Medical School, Kumamoto 860, Japan

Received September 5, 1991

Summary: In order to elucidate biosynthesis and secretion of natriuretic peptides in the early phase of acute myocardial infarction (AMI), we measured the plasma level of brain natriuretic peptide (BNP), a novel cardiac hormone secreted from the ventricle, in patients with AMI and compared with that of atrial natriuretic peptide (ANP). The plasma level of BNP increased rapidly (within hours from the onset of AMI) and markedly (>100 times the normal level) as compared to that of ANP. The plasma ANP level correlated with pulmonary capillary wedge pressure (PCWP), whereas the plasma BNP level did not correlate with PCWP but highly correlated inversely with cardiac index. These results indicate that BNP is secreted from the heart much more acutely and prominently than ANP in the early phase of AMI, in association with left ventricular dysfunction.

Brain natriuretic peptide (BNP) was first isolated from the porcine brain (1) as a possible neuropeptide, but subsequent studies have indicated that BNP is a cardiac hormone synthesized and secreted predominantly by the ventricle and constitutes a natriuretic peptide family involved in cardiovascular homeostasis together with atrial natriuretic peptide (ANP) derived mainly from the atrium (2-12). We previously demonstrated that, although the normal plasma level of BNP (0.9 fmol/ml) is below one-sixth of that of ANP (6.4 fmol/ml), the plasma BNP level increases much more markedly than the ANP level in chronic congestive

* To whom correspondence should be addressed.

Abbreviations: BNP, brain natriuretic peptide; ANP, atrial natriuretic peptide; AMI, acute myocardial infarction.

0006-291X/91 $1.50

heart failure (CHF), suggesting a discrete pathophysiological role of BNP in a dual cardiac natriuretic peptide system (8-10).

It has been reported that the plasma ANP level increases several-fold following acute myocardial infarction (AMI) both in humans (13) and in animal models (14), in close relation to atrial pressure (13) and infarct size (14). However, there have been some controversies on the change of the plasma ANP level during the very acute phase of AMI (15), and biosynthesis and secretion of natriuretic peptides in the early phase of AMI remain unclarified. In the present study, using specific radioimmunoassays (RIAs), we investigated the change in plasma levels of BNP and ANP in patients with AMI.

MATERIALS AND METHODS

Peptides: Synthetic human BNP (hBNP[77-108]) and [Tyr^{82}]-hBNP [83-108] were donated from Professor H. Matsuo at the National Cardiovascular Center, Suita, Japan. α-Human ANP (α-hANP, or human ANP[99-126]) was purchased from Peptide Institute, Inc., Minoh, Japan.

Plasma samples: Plasma samples were obtained from 13 consecutive patients (11 men, 2 women; aged 40 to 85 years) with AMI within 10 hours from the oneset of chest pain and thereafter every 4-24 hours over 4 days. Eight patients had anterior AMI, 4 inferior AMI and 1 posterolateral AMI. None had evidence of renal insufficiency. Blood was withdrawn from the antecubital or femoral vein in a recumbent position, immediately transferred to chilled siliconized glass tubes containing Na_2EDTA (1mg/ml) and aprotinin (1,000 KIU/ml, Ohkura Pharmaceutical, Kyoto, Japan), and centrifuged at 4°C. Plasma was immediately frozen and stored at -20°C until assay.

Hemodynamic and biochemical measurements: Hemodynamic measurements were performed throughout the course with the Swan-Ganz catheter placed immediately after admission in all patients. According to Forrester's classification (16), 7 patients were classified as subset I, 3 as subset II, 2 as subset III, and 1 as subset IV. Serum creatine kinase (CK) and CK-MB activities were measured by Autoanalyzer.

RIA for BNP: The RIA for BNP was performed as described previously in detail (10). In brief, [Tyr^{82}]-hBNP [83-108] was radioiodinated by the chloramine T method. The specific activity of [^{125}I][Tyr^{82}]-hBNP [83-108] ranged from 500 to 900 μCi/μg. The monoclonal antibody named KY-hBNP-I recognized the ring structure of hBNP. The cross-reactivity with α-hANP in this RIA was <0.005% on a molar basis. The minimal detectable concentration of BNP-like immunoreactivity (BNP-LI) in plasma was 10 fmol/ml.

RIA for ANP: The RIA for ANP was carried out as already reported (17,18). This RIA recognized the carboxy-terminal portion of α-hANP, α-ANP [17-28]. The cross-reactivity with hBNP was <0.01% on a molar basis.

Statistical analysis: Data were expressed as means ± SE. Statistical analysis was performed using Student's t test or Duncan's multiple range test when appropriate. Linear regression analysis was used to determine correlations between results.

RESULTS AND DISCUSSION

Serial dilution curves of plasma samples in the RIA for BNP were parallel to the standard curve of hBNP. Figure 1 shows time courses of plasma BNP-LI and ANP-LI levels and serum CK activity after onset of AMI in 4 representative cases with various degrees of severity. In patients of Forrester's subset I without heart failure, plasma BNP levels increased within 2-24 hours after onset, reached a peak value of at least 20 times the normal plasma BNP level around 24 hours after onset, and then declined gradually, as represented by Case 1 in Figure 1. In contrast, the plasma ANP level showed no or modest (within several-fold) increase. There was no significant difference in the peak value or time course of plasma BNP as to the site of infarction. The augmentation of the plasma BNP level was much more marked in patients with heart failure (Forrester's subsets II-IV). In Case 2, although there was a substantial elevation of the plasma ANP level during transient atrial fibrillation (Af), the plasma BNP level then began to increase and exceeded the ANP level as overt CHF developed. Other patients of Forrester's subset II also showed a considerable increase of plasma BNP. In patients of Forrester's subset III, the plasma BNP level showed a marked increase (200-300 times the normal level) with a rapid onset, although the plasma ANP level was only slightly higher than the normal range

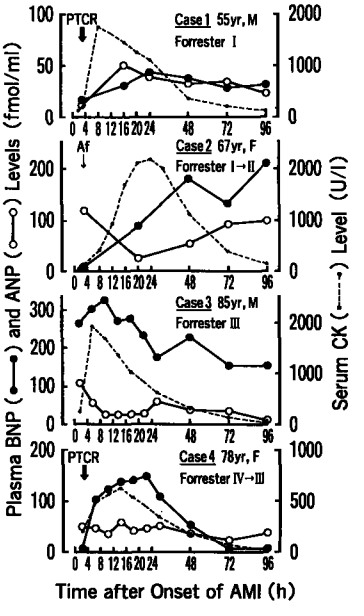

Figure 1. Plasma BNP, ANP, and serum CK levels in patients with AMI during the first 4 days after onset.

Table 1

Peak plasma BNP, ANP, and serum CK levels and hemodynamic parameters in patients with AMI

Forrester's subset	I	II	III or IV
No. of patients	7	3	3
BNP (fmol/ml)	64 ± 31	98 ± 45	233 ± 52*
ANP (fmol/ml)	39 ± 14	103 ± 22*	73 ± 18
CK (U/l)	1854 ± 389	2619 ± 229	1690 ± 545
CK-MB (U/l)	139 ± 46	214 ± 7	167 ± 56
PCWP (mmHg)	7.6 ± 1.2	19.3 ± 1.3**	12.3 ± 5.5
CI (l/min/m^2)	3.0 ± 0.1	3.2 ± 0.2	1.9 ± 0.2**,†

BNP, ANP, CK and CK-MB levels are represented by their peak values within 2 days from the onset of AMI. Pulmonary capillary wedge pressure (PCWP) and cardiac index (CI) were expressed by their representative values among multiple measurements during the first 2 days by which patients were thus classified.
Values are means ± SE.
* $p<0.05$, ** $p<0.01$ vs. values in subset I.
† $p<0.01$ vs. values in subset II.

(Case 3). In a patient of Forrester's subset IV (Case 4), the plasma BNP level also increased acutely and prominently during the first 24 hours, while the plasma ANP level remained almost constant.

Table 1 summarizes peak plasma BNP, ANP, and serum CK levels and hemodynamic parameters during the first 2 days. When compared with normal levels (8-10), the increase of the BNP level in patients of Forrester's subset I averaged 70 times, and was much more prominent than that of the ANP level (6 times). The peak BNP level was maximally elevated in patients of Forrester's subset III or IV, while the peak ANP level was the highest in those of subset II. The peak plasma ANP level correlated significantly with time-matched pulmonary capillary wedge pressure (PCWP) ($r = 0.58$, $p<0.05$), but not with cardiac index (CI) ($r = -0.16$). In contrast, the peak BNP level showed a highly inverse correlation with time-matched CI ($r = -0.81$, $p<0.01$), while it did not correlate with PCWP ($r = -0.25$). The peak BNP or ANP level did not show significant correlations with heart rate, blood pressure, peak CK or CK-MB level, or cummulative CK-MB release. These results indicate that the augmented plasma level of BNP is associated with impaired left ventricular performance, while that of ANP is related to increased cardiac filling pressure.

The present study demonstrates that the plasma level of BNP increases acutely and much more prominently than that of ANP in the early phase of AMI. Since BNP is secreted predominantly from the ventricle (6,10-12), being in contrast to ANP derived mainly from the atrium, it

is well conceivable that the plasma BNP level reflects left ventricular function more properly than ANP. The plasma BNP level was augmented more than 100 times the normal level in patients with heart failure following AMI, even in those with no or modest increase of the plasma ANP level. Our unpublished observations in rat AMI models show the results on both BNPmRNA in the ventricle and plasma BNP consistent with the result in the current study. These findings indicate that the regulation of synthesis and secretion of BNP in the heart of AMI differs from that of ANP. Rapid change of the BNP synthesis and secretion may, in part, be attributed to a unique structure of the BNP gene, having the AT-rich sequence implicated in mRNA instability in its 3'-untranslated region (19), which is lacking in the ANP gene.

Another important finding in this study is a notable increase of plasma BNP level in patients without heart failure (Forrester's subset I). This raises the possibility that the synthesis and secretion of BNP could be stimulated by myocardial necrosis and/or local mechanical stress on ventricular cardiocytes even when global hemodynamic parameters are within normal ranges, suggesting that BNP may serve as a useful marker of subclinical left ventricular dysfunction in AMI. Further studies are ongoing in our laboratory to clarify the difference in mechanisms of synthesis and secretion between BNP and ANP, and the pathophysiological role of increased plasma BNP in acute heart failure including AMI.

ACKNOWLEDGMENTS

We cordially thank Ms. A. Kibune and Ms. S. Takahashi for their secretarial assistance. This work was supported in part by research grants from the Japanese Ministry of Education, Science and Culture, the Japanese Ministry of Health and Welfare "Disorders of Adrenal Hormone" Research Committee, Japan, 1990, Japan Tobacco Inc., Yamanouchi Foundation for Research on Metabolic Disorders, the Cell Science Research Foundation, 1990, Uehara Memorial Foundation, and by research grants for cardiovascular diseases (63C-2 and 2A-3) from the Japanese Ministry of Health and Welfare.

REFERENCES

1. Sudoh, T., Kangawa, K., Minamino, N., and Matsuo, H. (1988) Nature 332: 78-81.
2. Saito, Y., Nakao, K., Itoh, H., Yamada, T., Mukoyama, M., Arai, H., Hosoda, K., Shirakami, G., Suga, S., Minamino, N., Kangawa, K., Matsuo, H., and Imura, H. (1989) Biochem. Biophys. Res. Commun. 158: 360-368.

3. Itoh, H., Nakao, K., Kambayashi, Y., Hosoda, K., Saito, Y., Yamada, T., Mukoyama, M., Arai, H., Shirakami, G., Suga, S., Yoshida, I., Inouye, K., and Imura, H. (1989) Biochem. Biophys. Res. Commun. 161: 732-739.
4. Kambayashi, Y., Nakao, K., Itoh, H., Hosoda, K., Saito, Y., Yamada, , T., Mukoyama, M., Arai, H., Shirakami, G., Suga, S., Ogawa, Y., Jougasaki, M., Minamino, N., Kangawa, K., Matsuo, H., Inouye, K., and Imura, H. (1989) Biochem. Biophys. Res. Commun. 163: 233-240.
5. Kambayashi, Y., Nakao, K., Mukoyama, M., Saito, Y., Ogawa, Y., Shiono, S., Inouye, K., Yoshida, N., and Imura, H. (1990) FEBS Lett. 259: 341-345.
6. Ogawa, Y., Nakao, K., Mukoyama, M., Shirakami, G., Itoh, H., Hosoda, K., Saito, Y., Arai, H., Suga, S., Jougasaki, M., Yamada, T., Kambayashi, Y., Inouye, K., and Imura, H. (1990) Endocrinology 126: 2225-2227.
7. Nakao, K., Itoh, H., Kambayashi, Y., Hosoda, K., Saito, Y., Yamada, T., Mukoyama, M., Arai, H., Shirakami, G., Suga, S., Jougasaki, M., Ogawa, Y., Inouye, K., and Imura, H. (1990) Hypertension 15: 774-778.
8. Mukoyama, M., Nakao, K., Saito, Y., Ogawa, Y., Hosoda, K., Suga, S., Shirakami, G., Jougasaki, M., and Imura, H. (1990) Lancet 335: 801-802.
9. Mukoyama, M., Nakao, K., Saito, Y., Ogawa, Y., Hosoda, K., Suga, S., Shirakami, G., Jougasaki, M., and Imura, H. (1990) N. Engl. J. Med. 323: 757-758.
10. Mukoyama, M., Nakao, K., Hosoda, K., Suga, S., Saito, Y., Ogawa, Y., Shirakami, G., Jougasaki, M., Obata, K., Yasue, H., Kambayashi, Y., Inouye, K., and Imura, H. (1991) J. Clin. Invest. 87: 1402-1412.
11. Hosoda, K., Nakao, K., Mukoyama, M., Saito, Y., Jougasaki, M., Shirakami, G., Suga, S., Ogawa, Y., Yasue, H., and Imura, H. (1991) Hypertension 17: 1152-1155.
12. Ogawa, Y., Nakao, K., Mukoyama, M., Hosoda, K., Shirakami, G., Arai, H., Saito, Y., Suga, S., Jougasaki, M., and Imura, H. (1991) Circ. Res. 69: 491-500.
13. Matsubara, H., Nishikawa, M., Umeda, Y., Taniguchi, T., Iwashita, T., Kurimoto, T., Yamane, Y., and Inada, M. (1987) Am. Heart J. 113: 1457-1462.
14. Mendez, R.E., Pfeffer, J.M., Ortola, F.V., Bloch, K.D., Anderson, S., Seidman, J.G., and Brenner, B.M. (1987) Am. J. Physiol. 253: H1449-H1455.
15. Wencker, M., Lechleitner, P., Dienstl, F., Hauptlorenz, S., and Puschendorf, B. (1987) Lancet i: 1369.
16. Forrester, J., Diamond, G., Chatterjee, K., and Swan, H. (1976) N. Engl. J. Med. 295: 1356-1362.
17. Nakao, K., Sugawara, A., Morii, N., Sakamoto, M., Suda, M., Soneda, J., Ban, T., Kihara, M., Yamori, Y., Shimokura, M., Kiso, Y., and Imura, H. (1984) Biochem. Biophys. Res. Commun. 124: 815-821.
18. Sugawara, A., Nakao, K., Morii, N., Yamada, T., Itoh, H., Shiono, S., Saito, Y., Mukoyama, M., Arai, H., Nishimura, K., Obata, K., Yasue, H., Ban, T., and Imura, H. (1988) J. Clin. Invest. 81: 1692-1970.
19. Kojima, M., Minamino, N., Kangawa, K., and Matsuo, H. (1989) Biochem. Biophys. Res. Commun. 159: 1420-1426.