Effects of Water Deprivation and Morphine Administration on Atrial Natriuretic Peptide mRNA Levels in Rat Auricles

Kiyoshi Fukui, Hiroshi Iwao, Akio Nakamura, Toshiaki Tamaki and Youichi Abe

Department of Pharmacology, Kagawa Medical School, Kagawa 761-07, Japan

Received February 15, 1991 Accepted May 31, 1991

ABSTRACT — We investigated the influences of two potent stimuli, water deprivation (5 days) and morphine administration (100 mg/kg), on the level of atrial natriuretic peptide (ANP) mRNA in the rat auricles. The ANP mRNA level was measured by Northern blot hybridization analysis. The plasma concentration of ANP decreased in water deprived rats, and the ANP mRNA levels in both auricles of these rats were lower than those of the control, particularly in the left auricle. Thirty minutes after the injection of morphine, the plasma concentration of ANP markedly increased, while morphine increased the right auricular ANP mRNA level 4 hr after the administration. These data suggest that these stimuli can change ANP gene expression in the auricles and that the changes are induced differentially in both auricles.

Atrial natriuretic peptide (ANP) is a peptide hormone synthesized in the heart that influences circulatory homeostasis through its potent vasoactive and natriuretic activities (1). The gene structure for ANP has been elucidated, and its expression has been reported in the atrium, ventricle, aorta, and other tissues (2-7). Although many reports have shown that ANP can be released in response to atrial distention, volume expansion, tachycardia and some vasoconstrictors (8-11), there have been only a few reports showing the stimulus-induced change of ANP gene expression in atria (4, 12, 13).

Recent studies have demonstrated that the administration of a large dose of morphine induces a dramatic increase in plasma concentration of ANP, indicating markedly enhanced release (14, 15). Alternatively, it has been shown that water deprivation induces a reduction in the plasma ANP concentration and atrial ANP mRNA (2, 3).

The purpose of the present study is to examine if ANP gene expression in the auricle can be changed by these potent stimuli, morphine injection and water deprivation, and to characterize the change of ANP gene expression separately in the right and left auricles.

MATERIALS AND METHODS

Adult male Sprague-Dawley rats (250–300 g. body weight) were used. They were fed ad libitum standard laboratory chow and tap water for two weeks before the experiments. Experiments were performed according to the following protocols.

Effects of water deprivation: Two groups of rats (5 in each group) were used. The control group received water ad libitum, but the experimental group was deprived of water for 5 days.

Effects of morphine administration: Three groups of rats (6 in each group) were used.
Group 1: Untreated control. Group 2: Morphine hydrochloride was injected (100 mg/kg, s.c.), and the rats were decapitated 30 min after injection. Group 3: Morphine hydrochloride was injected (100 mg/kg, s.c.), and tracheal intubation was performed 15 min after injection. Rats were mechanically ventilated (3 ml/time, 85 times/min) from 30 min to 4 hr after the injection and then decapitated. All the rats used were killed by decapitation. Blood was collected into siliconized glass beakers containing EDTA-Na₂ (1 mg/ml), aprotinin (1000 kallikrein inactivator units/ml), and soybean trypsin inhibitor (100 units/ml); and the plasma was obtained by centrifugation. Right and left auricles were excised from the heart, rapidly frozen with liquid nitrogen, and stored at −80°C. The stored auricles were gathered in batches and subjected to extraction of total RNA. We repeated the experiments twice, and the results were similar; The results of one experiment are shown in the present paper.

Extraction of ANP from plasma samples was performed by the methods of Lang et al. (8), using a Sep-Pak C₁₈ cartridge. The ANP radioimmunoassay was performed with a commercially available kit (RPA512, Amersham).

Total RNA was extracted from the auricles using the methods of Chirgwin et al. (16) and quantified spectrophotometrically by absorption at 260 nm. The amounts of total RNA obtained per gram of each tissue were 0.96 ± 0.06 mg. Quantitative measurement of ANP mRNA was performed by Northern blot hybridization analysis as previously reported (17). In short, total RNA was denatured with 1 M glyoxal and 50% dimethylsulfoxide, electrophoresed on 1.5% agarose gels, and transferred to Biodyne A membranes (Pall Ultrafine Filtration Corp., Glen Cove, NY, U.S.A). We checked the integrity of the RNA on the ethidium bromide-stained agarose gel. The 368-base pair Hinc II and Stu I fragment derived from the cDNA insert of the clone prAFS-1 was labeled with ³²P by nick translation and used as a hybridization probe (2). After autoradiography, the density of the hybridization image was plotted against the amounts of applied total RNA, and regression lines were calculated using the least squares method. ANP mRNA levels were quantified from the slopes of the regression lines.

The values presented are means ± S.E. Student's t-test was used only when two groups of data were compared. A one-way analysis of variance followed by Dunnett's test was used for comparison among the plasma ANP concentrations in experiment 2 (effects of morphine). P values less than 0.05 were accepted as indicating statistically significant differences.

RESULTS

Table 1 shows the effects of water deprivation and morphine treatment on the plasma concentration of ANP. When the rats were deprived of water for 5 days, the plasma concentration of ANP was significantly reduced. Administration of morphine resulted in a marked increase in the plasma concentration of ANP at 30 min. The plasma concentration of ANP returned to the control level 4 hours after the administration of morphine.

Figure 1 is an autoradiogram obtained by Northern blot hybridization. ANP mRNA had

| Table 1. Effects of water deprivation and morphine administration on the plasma concentration of ANP |
|---------------------------------------------------------------|
|                  | Plasma conc.      |
|                  | (pg/ml)           |
| Control          |                  |
| Water deprivation|                  |
| Control          | (n = 5)           | 153 ± 8          |
| Morphone 30 min  | (n = 5)           | 112 ± 3*         |
| 4 hr             | (n = 6)           | 138 ± 17         |
|                  | (n = 6)           | 1212 ± 465*      |
|                  | (n = 6)           | 136 ± 13         |

Water deprivation for 5 days reduced the plasma concentration of ANP. Morphine (100 mg/kg, s.c.) increased the plasma concentration of ANP 30 min after administration. Asterisks denote a significant difference (P < 0.05) when compared with the control.
a single band of −950 nucleotides, which was similar to that reported earlier (2, 3). In the right auricles of water deprived rats, the ANP mRNA level decreased to one half of the control level, while on the left side, it decreased to one tenth (Figs. 1 and 2). Administration of morphine did not change the ANP mRNA level at 30 min (data not shown), but it induced a two-fold increase in the right auricular ANP mRNA level 4 hr after the administration (Fig. 3). There was no change in the ANP mRNA level of the left auricle 4 hr after the morphine injection.

DISCUSSION

By examining the effects of two powerful and alternative stimuli on ANP release, we have characterized ANP gene expression in the right and left auricle.

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Fig. 1. Northern blot hybridization analysis of total RNA from the auricles of control and water-deprived rats. A: control rats, left; B: control rats, right; C: water-deprived rats, left; D: water-deprived rats, right. Amounts of total RNA applied in each lane were 0.5, 1.0, 1.5 and 2.0 μg, respectively. ANP mRNA showed a single band of −950 nucleotides. Water deprivation for 5 days reduced the auricular ANP mRNA level. The change in the left auricles was more marked than that in the right auricles.
Fig. 2. Regression analysis plots of auricular ANP mRNA in the control and water-deprived rats using radiodensitometric Northern blot hybridization analysis. ANP mRNA levels were quantified from the slopes of the regression lines. Water deprivation reduced the ANP mRNA level to one tenth in the left auricles and to one half in the right auricle. Left panel (Left auricles): control (○), \( y = 663x - 11, r = 0.998; \) 5 days after water deprivation (●), \( y = 61x - 12, r = 0.971. \) Right panel (Right auricles): control (○), \( y = 515x - 18, r = 0.994; \) 5 days after water deprivation (●), \( y = 205x + 51, r = 0.999. \)

Fig. 3. Regression analysis plots of auricular ANP mRNA in the control and morphine-treated rats using radiodensitometric Northern blot hybridization analysis. ANP mRNA levels were quantified from the slopes of the regression lines. Administration of morphine induced a two-fold increase in ANP mRNA level only in the right auricles. Left panel (Left auricles): control (○), \( y = 202x - 45, r = 0.967; \) 4 hr after morphine administration (▲), \( y = 256x - 35, r = 0.987. \) Right panel (Right auricles): control (○), \( y = 217x - 27, r = 0.991; \) 4 hr after morphine administration (▲), \( y = 431x - 47, r = 0.990. \)
Severe dehydration suppressed the ANP mRNA level markedly, while the plasma levels fell only slightly. These findings indicate a greater inhibition of synthesis than secretion and essentially confirm the results of Takayanagi et al. (3). In our experiments, ANP mRNA levels of the right and left auricle did not change in parallel. The left auricle showed a greater change in mRNA than the right auricle. The reasons for this are unclear, but probably reflect a greater inhibition of synthesis in the left auricle.

Administration of morphine increased plasma levels dramatically at 30 min, but this increase was coupled with insignificant changes in mRNA. Since the content of ANP in the auricle is 103-fold the amount of ANP in one milliliter of plasma (18, 19), the release of a small portion of the ANP store may possibly lead to a rise of the plasma concentration, and the application of the stimulus may not affect the mRNA synthesis within a short period. However, 4 hr after injection, the ANP mRNA level increased, and the plasma level of ANP had returned to the control level, indicating that the earlier enhanced secretion could not be sustained and that delayed influence on the mRNA level may be occurred.

Gutkowska et al. (15) implied that there is an opioid receptor mechanism involved in the morphine-induced increase in plasma ANP concentration. However, we have confirmed in separate experiments that large doses of morphine kill all animals within 40 min due to respiratory depression. On the other hand, it has been reported that exposure of animals to chronic or acute hypoxia increases plasma ANP concentration up to 2–10-fold (20, 21) and that chronic hypoxia decreases the left atrial ANP mRNA level (13). Although the mechanism of hypoxia-induced release of ANP is unclear, pulmonary hypoxic vasoconstriction and change in the pulmonary circulation may be implied (22–24). From these findings, we consider that the morphine-induced increase in plasma ANP concentration is most likely related to respiratory depression and possibly a change in pulmonary circulation, and that increased ANP release may secondarily enhance the ANP gene expression in the right auricle.

In conclusion, potent stimuli that affect the release of ANP can also change ANP gene expression, and the changes occur differentially between both auricles.

Acknowledgments
We thank Dr. S. Skinner for helpful advice for preparing this manuscript. We are grateful to Dr. S. Nakanishi (Institute for Immunology, Faculty of Medicine, Kyoto University) for giving us the clone prAFS-1. This work was supported by Grant-in-Aid for Scientific Research 63770134 from the Ministry of Education, Science and Culture of Japan.

REFERENCES
1 Sagnella, G.A. and MacGregor, G.A.: Cardiac peptides and the control of sodium excretion. Nature 299, 666–667 (1984)
2 Nakayama, K., Ohkubo, H., Hirose, T., Inayama, S. and Nakanishi, S.: mRNA sequence for human cardiodilation-atrial natriuretic factor and regulation of precursor mRNA in rat atria. Nature 310, 699–701 (1984)
3 Takayanagi, R., Tanaka, I., Maki, M. and Inagami, T.: Effects of changes in water-sodium balance on levels of atrial natriuretic factor messenger RNA and peptide in rats. Life Sci. 36, 1843–1848 (1985)
4 Lattion, A.-L., Michel, J.-B., Arnauld, E., Corvol, P. and Sonbriener, F.: Myocardial recruitment during ANF mRNA increase with volume overload in the rat. Am. J. Physiol. 251, H890–H896 (1986)
5 Gardner, D.G., Heine, S., Trachewsky, D., Schenck, D. and Baxter, J.D.: Atrial natriuretic peptide mRNA is regulated by glucocorticoids in vivo. Biochem. Biophys. Res. Commun. 139, 1047–1054 (1986)
6 Gardner, D.G., Deshepper, C.F. and Baxter, J.D.: The gene for the atrial natriuretic factor is expressed in the aortic arch. Hypertension 9, 103–106 (1987)
7 Takayanagi, R., Imada, T. and Inagami, T.: Synthesis and presence of atrial natriuretic factor in rat ventricle. Biochem. Biophys. Res. Commun. 142, 483–488 (1987)
8 Lang, R.E., Tholken, H., Ganten, D., Luit, F.C., Ruskoaho, H. and Unger, Th.: Atrial natriuretic factor—a circulating hormone stimulated by volume loading. Nature 314, 264–266 (1985)
9 Dietz, J.R.: Control of atrial natriuretic factor release from a rat heart-lung preparation. Am. J. Physiol. 252, R498–R502 (1987)
10 Schiebinger, R.J. and Linden, J.: Effect of atrial contraction frequency on atrial natriuretic peptide secretion. Am. J. Physiol. 251, H1095–H1099 (1986)
11 Katsube, N., Schwartz, D. and Needleman, P.: Release of atriopeptin in the rat by vasoconstrictors or water immersion correlates with changes in right atrial pressure. Biochem. Biophys. Res. Commun. 133, 937–944 (1985)
12 Lattion, A.-L., Aubert, J.F., Fluckiger, J.P., Nussberger, J., Waeder, B. and Brunner, H.R.: Effect of sodium intake on gene expression and plasma levels of ANF in rats. Am. J. Physiol. 255, H245–H249 (1988)
13 Stockmann, P.T., Will, D.H., Sides, S.D., Brunner, S.R., Wilner, G.D., Leahy, K.M., Wiegand, R.C. and Needleman, P.: Reversible induction of right ventricular atriopeptin synthesis in hypertrophy due to hypoxia. Circ. Res. 63, 207–213 (1988)
14 Horoky, K., Gutkowska, J., Garcia, R., Thibault, G., Genest, J. and Cantin, M.: Effect of different anesthetics on immunoreactive atrial natriuretic factor concentrations in rat plasma. Biochem. Biophys. Res. Commun. 129, 651–657 (1985)
15 Gutkowska, J., Racz, K., Garcia, R., Thibault, G., Kuchel, O., Genest, J. and Cantin, M.: The morphine effect on plasma ANF. Eur. J. Pharmacol. 131, 91–94 (1986)
16 Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J.: Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18, 5294–5299 (1979)
17 Fukui, K., Iwao, H., Nakamura, A., Yamamoto, A., Tamaki, T., Shoji, T., Kimura, S., Aki, Y., Hasui, K., Ohkubo, H., Nakanishi, S. and Abe, Y.: Captopril and hydralazine suppress atrial natriuretic peptide (ANP) gene expression in the ventricles of spontaneously hypertensive rat. Biochem. Biophys. Res. Commun. 160, 310–316 (1989)
18 Morii, N., Nakao, K., Kihara, M., Sugawara, A., Sakamoto, M., Yamori, Y. and Imura, H.: Decreased content in left atrium and increased plasma concentration of atrial natriuretic polypeptide in spontaneously hypertensive rats (SHR) and SHR stroke-prone. Biochem. Biophys. Res. Commun. 135, 74–81 (1986)
19 Ruskoaho, H. and Leppaluoto, J.: Immunoreactive atrial natriuretic peptide in ventricles, atria, hypophalamus, and plasma of genetically hypertensive rats. Circ. Res. 62, 384–394 (1988)
20 McKenzie, J.C., Tanaka, I., Inagami, T., Misono, K.S. and Klein, R.M.: Alterations in atrial and plasma atrial natriuretic factor (ANF) content during development of hypoxia-induced pulmonary hypertension in the rat. Proc. Soc. Exp. Biol. Med. 181, 459–463 (1986)
21 Baertschi, A.J., Adams, J.M. and Sullivan, M.P.: Acute hypoxemia stimulates atrial natriuretic factor secretion in vivo. Am. J. Physiol. 255, H295–H300 (1988)
22 Nagasaka, Y., Bhattacharya, J., Gropper, M.A. and Staub, N.C.: Micropuncture measurement of lung microvascular pressure profile during hypoxia in cats. Fed. Proc. 42, 595 (1983)
23 Marshall, C. and Marshall, B.E.: Influence of perfusate PO2 on hypoxic pulmonary vasoconstriction in rats. Circ. Res. 52, 691–696 (1983)
24 Brashers, V.L., Peach, M.J. and Rose, C.E., Jr.: Augmentation of hypoxic pulmonary vasoconstriction in the isolated perfused rat lung by in vitro antagonists of endothelium-dependent relaxation. J. Clin. Invest. 82, 1495–1502 (1988)