Effect of α -human Atrial Natriuretic Polypeptide on Anterior Pituitary Function in Men

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Abstract

In order to examine the effects of α -human atrial natriuretic polypeptide (α -hANP) on the basal plasma concentrations of GH, TSH, LH, FSH and PRL in humans, synthetic α -hANP was infused into 10 normotensive, euvoletic, healthy volunteers. There were observed marked hypotensive, diuretic and natriuretic effects during the α -hANP infusion. The basal plasma concentrations of GH, TSH, LH and FSH, showed no significant change following the α -hANP infusion. However, significant suppression of the plasma PRL concentration was observed with the α -hANP administration. The mean plasma PRL concentration tended to be decreased during 20 min of α -hANP infusion, however, there the differences were not statistically significant. A significant reduction in the mean plasma PRL concentration (-20% , $P < 0.5$) was observed 10 min after the end of infusion, following the reversion to the preinfusion level at 70 min after the end of infusion. Such a significant and delayed suppression was not seen in the case of placebo infusion. The data suggest that the circulating hANP may reduce the release of PRL.

Atrial natriuretic polypeptides (ANP) are a group of peptides purified from human and rat atrial tissues and shown to be involved in circulatory homeostasis (deBold *et al.*, 1981; Kangawa and Matsuo, 1984;

Kangawa *et al.*, 1984). Immunohistochemical studies using a specific antiserum to α -ANP (composed of 28 amino acids) have demonstrated that immunoreactive ANP containing neurons were present in the hypothalamus, pituitary and pons of rats (Saper *et al.*, 1985; MacKenzie *et al.*, 1985; Kawata *et al.*, 1985). Also, specific binding sites for ANP have been observed in the rat olfactory bulb, subfornical organ, median eminence and pituitary gland (Quirion *et al.*, 1984).

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Taken together, this evidence suggests that ANP may act as a central neurotransmitter or neuromodulator as well as peripheral hormone in the regulation of water and electrolyte homeostasis. In this communication, we have attempted to examine the effects of α -hANP on the plasma concentrations of five pituitary hormones in humans.

Materials and Methods

1. Subjects

The subjects were normotensive Japanese adult volunteers, 8 males aged 41.3 ± 4.6 (21–55 yo) and 2 females (19 and 27 y) for α -hANP infusion, and four men aged 25.2 ± 2.2 (range 23–28 years) and four women aged 20.2 ± 1.0 (19–21 years) for placebo infusion. All subjects were free from endocrine, liver, kidney and heart disease and were fed on a regular diet served in Kyushu University Hospital, which contained 2370 kcal and 12 g of NaCl (Na ion: 200 mEq) per day for one week before initiation of the test. The body weight of each subject was within 5% of the ideal body weight. Informed consent was obtained from each volunteer.

2. α -hANP

Synthetic α -hANP was obtained from the Peptide Institute (Osaka, Japan). The purity of the peptide was checked by high-performance liquid chromatography before use. The lyophilized peptide (500 μ g) was dissolved in 5 ml of physiological saline and passed through a Millex-GV filter (0.22 μ m) (Millipore Corporation, Bedford, MA, USA) for sterilization. Usually such preparations were tested within two weeks after the peptide solutions were made.

3. Infusion Study

The subjects were kept fasting, voided completely and drank 200 ml of distilled water at 0800 h. After one hour urine was collected, 200 ml of water was drunk and α -hANP was infused intravenously at the rate of 0.1 μ g/kg/min for 20 min (total infusion volume; 40 ml). Forty ml of physiological saline was infused as the placebo infusion. The blood pressure and pulse rate were monitored continuously using an automatic sphygmomanometer (Nippon Kolin Co. Tokyo, Japan) during the study. At 1000 h, the

urine was collected again and 200 ml of drinking water was given to each subject. Neither complication nor allergic reaction was observed during α -hANP infusion. Blood was drawn into a tube containing EDTA-2Na and Trasylol (500 KIU/ml, Bayer, Leverkusen, West Germany), centrifuged at $5,000 \times g$ for 10 min and the plasma sample was stored at -20°C for hormone assay. The urine samples collected at the intervals of 60 min were also stored at -20°C .

4. Assay

Plasma samples were assayed for PRL, GH, LH, FSH and TSH. All anterior pituitary hormones were measured by standard double antibody RIA systems. PRL was assayed by the method of Yoshida *et al.* (1983). The detected lower limit of the PRL assay was 250 pg/tube. The intra and interassay coefficients of variation were 8% and 13%, respectively. GH was assayed by the method of Yoshida *et al.* (1979). LH and FSH were assayed by the method of Okamura *et al.* (1980). TSH was assayed by the method of Azukizawa *et al.* (1975).

5. Statistics

The data obtained are expressed as the mean $\pm$ SEM, and Student's independent *t*-test was used for determination of significance. $P < 0.05$ was considered as of statistical significance.

Results

The mean systolic and diastolic pressure promptly declined to 10 and 5 mmHg below the pretreatment level following α -hANP infusion and subsequently returned back to the level before infusion. Table 1 summarizes

Table 1. Effects of synthetic α -hANP on renal functions in normal subjects

	1	2	3
urine volume (ml/hr)	119 $\pm$ 37	415 $\pm$ 49**	169 $\pm$ 26
urinary sodium excretion (mmol/hr)	8.1 $\pm$ 1.5	14.7 $\pm$ 2.3**	14.1 $\pm$ 2.4*

1. before infusion; 2. during infusion; 3. after infusion * $P < 0.05$, * $P < 0.01$ compared with 1

the diuretic and natriuretic effects induced by α -hANP infusion in all subjects. The urine volume and urinary sodium excretion revealed 3.5 fold and 1.8 fold increases in the preinfusion level. The effect on the urinary sodium excretion was more prolonged than that on urine output. Plasma GH, TSH, LH and FSH showed no significant changes during α -hANP infusion, while significant suppression was found in the level of plasma PRL (Table 2). Fig. 1 shows the suppression of plasma PRL concentrations with

α -hANP infusion. The mean plasma PRL concentration showed no significant decrease during the period of infusion, but apparent suppression was evident at 10 min after the end of infusion (-25% of the preinfusion level, $P<0.01$). The significant decrease was continued until 40 min after the end of infusion ($P<0.05$). Thereafter, the mean plasma PRL concentration reverted to the level of placebo infusion at 70 min after the end of infusion. Although there were marked variations in the age of the subject

Table 2. Effects of synthetic α -hANP on plasma GH, TSH, LH, FSH and PRL concentrations in normal human subjects

Hormones	Unit	0	5	10	15	20	30	45	60	120	(min)
GH	ng/ml	4.8	6.7	5.8	6.0	5.1	4.5	4.1	2.7	2.7	2.6*
		± 1.4	± 2.0	± 2.4	± 2.5	± 1.7	± 1.6	± 1.3	± 0.8	± 0.6	± 0.6
TSH	μ U/ml	7.6	6.5	6.1	8.6	10.4	6.9	10.3	7.6	6.1	7.1
		± 0.6	± 0.9	± 1.5	± 1.8	± 2.3	± 1.4	± 2.7	± 1.1	± 0.8	± 1.3
LH	mIU/ml	19.8	18.7	19.4	18.5	18.6	18.2	18.8	19.3	19.9	18.6
		± 2.8	± 2.5	± 2.5	± 2.4	± 2.8	± 2.9	± 2.4	± 2.2	± 2.8	± 2.6
FSH	mIU/ml	10.1	9.9	9.9	9.9	9.0	9.4	8.3	10.1	9.8	10.5
		± 1.2	± 1.0	± 1.1	± 1.4	± 1.5	± 1.3	± 1.6	± 1.8	± 1.8	± 1.8
PRL	ng/ml	23.0	24.8	24.2	22.2	21.6	17.9**	17.2*	16.4*	22.3	23.5
		± 6.6	± 7.6	± 7.6	± 7.2	± 7.0	± 5.5	± 4.6	± 4.0	± 7.9	± 8.2

** $P<0.01$, * $P<0.05$ compared with the level in preinfusion (Student's t -test)

Fig. 1. Time-course of the effect of synthetic α -hANP on the plasma PRL concentration. α -hANP was administered i.v. at $0.1 \mu\text{g/kg}$ per min for 20 min (●). Physiological saline was infused i.v. as a control (○). The shaded area indicates the α -hANP or saline infusion period. The mean plasma concentration before infusion is represented as 100%. Values are means $\pm$ S.E.M., ** $P<0.01$, * $P<0.05$ compared with the level in preinfusion (Student's t -test).

and the basal level of plasma PRL, the rate of suppression was not significantly correlated with these parameters (data not shown). The placebo infusion did not suppress the plasma PRL concentration in all subjects.

Discussion

It is presumed that ANP may modulate the synthesis and secretion of the anterior and posterior pituitary hormones, because a considerable amount of immunoreactive ANP is present in the hypothalamus and pituitary gland in rat (Tanaka *et al.*, 1984; Saper *et al.*, 1985; MacKenzie *et al.*, 1985; Kawata *et al.*, 1985). Also, it was observed that ANP could stimulate guanylate cyclase activity and conversely, suppress adenylate cyclase activity in rat pituitary (Anand-Srivastava *et al.*, 1985; Heisler *et al.*, 1986). Very recently, we obtained evidence that α -hANP administration (0.1 μ g/kg/min, 20 min) caused significant suppression of the basal plasma AVP concentration in human (Fujio *et al.*, 1986). However, the same infusion of α -hANP had no effect on the plasma ACTH concentration (Ohashi *et al.* 1986).

In this study, we observed that the basal plasma PRL concentration was also suppressed by the α -hANP infusion. It is known that water intake itself causes a reduction in the plasma PRL concentration (Buckman *et al.*, 1973). However, this possibility could be neglected because the placebo infusion failed to induce a change in any of the subjects. Another consideration is that the decrease in the circulatory volume caused by α -ANP infusion, may modulate the PRL secretion, because there was no significant diuresis in the cases subjected to the placebo study (data not shown). However, it was reported that the reduction in the circulatory volume raised the plasma PRL concentration in rat (Boehm *et al.*, 1982). The mechanism of the inhibitory effect of α -

hANP on the plasma PRL concentration is unknown. Preliminary evidence has been obtained showing that synthetic α -hANP suppressed the angiotensin II-induced release of PRL from cultured rat anterior pituitary cell (M. Ohashi *et al.*, unpublished observation). The significant reduction in plasma PRL may account for the antagonistic effects on angiotensin II by ANP (Currie *et al.*, 1983). However, it remains to be established whether α -hANP-induced suppression may have some physiological meaning regarding the regulation of PRL secretion, because of the pharmacological doses of synthetic α -hANP required to elicit such an effect.

In conclusion, significant and delayed suppression was seen in the plasma PRL concentration following synthetic α -hANP infusion, but the plasma GH, TSH and FSH concentrations were not changed.

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