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ATRIAL NATRIURETIC POLYPEPTIDE IN SPINAL CORD AND AUTONOMIC GANGLIA

Narito Morii[1], Kazuwa Nakao[1,2*], Hiroshi Itoh[1], Shozo Shiono[1], Takayuki Yamada[1], Akira Sugawara[1], Yoshihiko Saito[1], Masashi Mukoyama[1], Hiroshi Arai[1], Makoto Sakamoto[1], and Hiroo Imura[1]

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

[2] Radioisotope Research Center, Kyoto University, Kyoto, Japan.

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Using a radioimmunoassay for α-rat atrial natriuretic polypeptide (α-rANP), tissue levels of α-rANP-like immunoreactivity (-LI) in the rat spinal cord and autonomic ganglia were investigated. The α-rANP-LI level was higher in the more caudal parts of the spinal cord and the highest in the sacral spinal cord. α-rANP-LI was also detected in the superior cervical and coeliac ganglia. Gel permeation chromatographic analysis showed that the major peak of α-rANP-LI in the spinal cord was a low molecular weight form co-eluted with synthetic α-rANP. Reverse-phase high performance liquid chromatographic analysis revealed that α-rANP-LI with a low molecular weight in the spinal cord consisted of several components, two major components of which co-migrated with synthetic α-rANP(4-28) and α-rANP(5-28), whereas little immunoreactivity was eluted at the position of α-rANP. These findings suggest the involvement of ANP in the function of the spinal cord and autonomic nervous system.

Atrial natriuretic polypeptide (ANP), a family of vasoactive peptides with potent diuretic, natriuretic and vasorelaxant activities, was isolated from human and rat atrial tissues (1-4) and implicated in fluid homeostasis and blood pressure control as a circulating hormone.

*Address correspondence to: Kazuwa Nakao, M.D., Ph.D., Second Division, Department of Medicine, Kyoto University School of Medicine, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606, Japan.

Abbreviations: ANP, atrial natriuretic polypeptide; α- and γ-rANP, α- and γ-rat atrial natriuretic polypeptide; -LI, -like immunoreactivity; RIA, radioimmunoassay; HP-GPC, high performance gel permeation chromatography; RP-HPLC, reverse-phase high performance liquid chromatography.

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Using radioimmunoassay (RIA) and histochemistry, we demonstrated the widespread distribution of α-rat ANP-like immunoreactivity (α-rANP-LI) in the rat brain (5-8). The α-rANP-LI level is the highest in the hypothalamus and septum (5). ANP containing cells and fibers are most prevalent in the periventricular tissues surrounding the third ventricle (7,8), which are areas known to be involved in central cardiovascular control. ANP is also present in the human and monkey brains (9). Gel chromatographic analysis revealed that the predominant form of ANP is a low molecular weight form in the brain (5,6,10). The major components with a low molecular weight are α-rANP(4-28) and α-rANP(5-28) in the rat brain and different from the circulating form, α-rANP with 28 amino acids (10). We also reported that responses of the tissue ANP level to water deprivation or sodium loading are different between the brain and the heart (6). Recently, we observed that the intracerebroventricular (icv) application of ANP inhibits water drinking (11-14), salt appetite (15), pressure response to angiotensin II (16,17) and the release of antidiuretic hormone (18) and ACTH (19). We also found that icv injection of ANP decreases tissue levels of dopamine and DOPAC in the hypothalamus and septum (20). These findings suggest that brain ANP plays a role in the control of fluid and electrolyte balance and blood pressure regulation as a neuropeptide.

However, little is known about ANP in the spinal cord. In this study, we investigated the distribution of ANP in the rat spinal cord and sympathetic ganglia.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 300-350 g were used. They were housed in a light (12 hr light/12 hr dark cycle), temperature and humidity controlled room, and fed regular rat chow pellets (0.5 % NaCl, 0.84 % KCl) and tap water ad libitum. All rats were killed by decapitation.

Tissue dissection and extraction procedure

Spinal cords (n=6) were removed immediately after decapitation, separated into the cervical, upper thoracic, lower thoracic, lumbar

and sacral parts by dissecting near levels of Th1, Th6, L1 and S1 using spinal nerves as landmarks. Superior cervical ganglia and coeliac ganglia were pooled from 30 rats. Extraction of tissues was performed as previously reported (5). In brief, tissues were boiled for 5 minutes in 10 volumes of 0.1 M acetic acid and homogenized with a polytron homogenizer for 60 seconds. The homogenate was centrifuged at 30,000 x g for 30 minutes at 4°C and the supernatant was used for assay.

Peptides

α-rANP (cardionatrin), α-rANP(3-28) (ANF(8-33)), α-rANP(4-28) (ANF IV or auriculin B), α-rANP(5-25) (atriopeptin I), α-rANP(5-27) (atriopeptin II) and α-rANP(5-28) (atriopeptin III) were purchased from Peninsula Laboratory, Belmont, CA.

RIA

α-rANP-LI in extracts was measured by a RIA as previously reported (21). The RIA showed 100 % cross-reactivity with α-rANP(3-28), α-rANP(4-28) and α-rANP(5-28), and 11.0 % cross-reactivity with α-rANP(5-27) on a molar basis. Cross-reactivities with α-ANP(1-6), α-hANP(8-22) and α-rANP(24-28) were less than 0.2 %.

High performance gel permeation chromatography (HP-GPC)

HP-GPC were performed on a TSK-GEL G2000 SW column (7.8 x 600 mm, Toyo Soda, Tokyo, Japan) as previously reported (5,21). The column was calibrated with a polypeptide molecular weight calibration kit (Pharmacia, Uppsala, Sweden) and synthetic α-rANP.

Reverse-phase high performance liquid chromatography (RP-HPLC)

RP-HPLC was carried out on a TSK-GEL ODS 120T column (4.6 x 75 mm, Toyo Soda, Tokyo, Japan) as previously reported (10). The column was eluted at 0.6 ml/minute with 0.08 % trifluoroacetic acid with linear gradients of acetonitrile as follows: 20.5 % to 23.0 % in 55 minutes and 23.0 to 60.0 % over subsequent 30 minutes. The retention times of α-rANP (5-25), α-rANP(5-27), α-rANP(3-28), α-rANP(4-28), α-rANP and α-rANP(5-28) were 6.0, 15.0, 20.8, 24.7, 27.2 and 30.1 minutes, respectively.

RESULTS

Table 1 shows the concentrations of α-rANP-LI in the spinal cord, and superior cervical and coeliac ganglia in rats. α-rANP-LI was detected throughout the spinal cord. The α-rANP-LI level was higher in the more caudal parts of the spinal cord and the highest in the

Table 1. α-rANP-like immunoreactivity in the rat spinal cord and autonomic ganglia

Regions	α-rANP-LI (ng/g wet tissue)
Spinal cord	
Cervical	1.93 ± 0.12
Upper thoracic	2.07 ± 0.32
Lower thoracic	2.40 ± 0.24
Lumbar	3.36 ± 0.62
Sacral	7.94 ± 1.46
Autonomic ganglia	
Cervical ganglion	0.89
Coeliac ganglion	3.08

Values are means ± SEM.

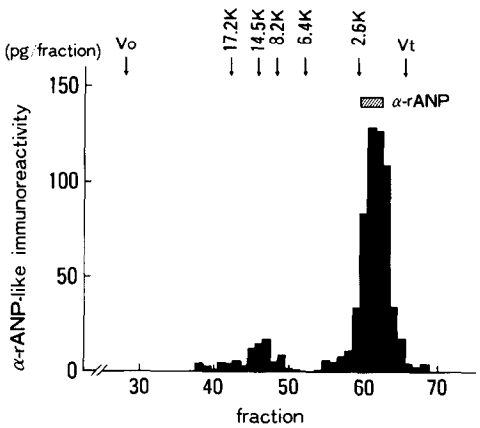

Fig. 1. A gel filtration pattern of the extract from the rat spinal cord on a TSK-GEL G 2000 SW column (7.5 x 600 mm). Arrows denote the elution positions of a series of myoglobins of the polypeptide molecular weight calibration kit, void volume (Vo) and total volume (Vt).

sacral spinal cord. α-rANP-LI was also detected in the superior cervical and coeliac ganglia, and the concentrations in these sympathetic ganglia were comparable to that in the spinal cord. Figure 1 illustrates the result of HP-GPC analysis with α-rANP-LI in the rat spinal cord. α-rANP-LI in the spinal cord was composed of high and low molecular weight forms. The major component was a low molecular

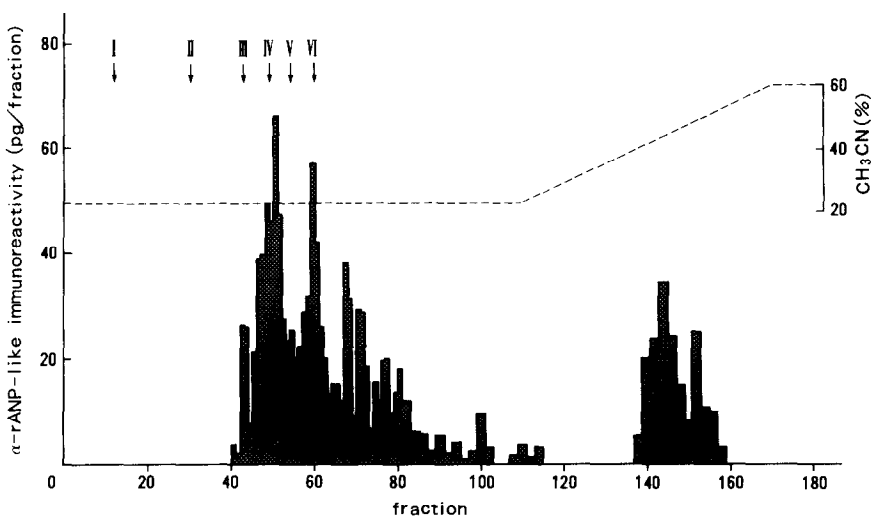

Fig. 2. A reverse-phase high performance liquid chromatographic profile of the extract from the spinal cord on a TSK-GEL ODS 120T column (4.6 x 75 mm). Arrows denote the elution positions of synthetic α-rANP and its related peptides. I, α-rANP(5-25); II, α-rANP(5-27); III, α-rANP(3-28); IV, α-rANP(4-28); V, α-rANP; and VI, α-rANP(5-28).

weight form co-eluted with synthetic α-rANP. A small amount of α-rANP-LI was eluted at the position corresponding to γ-rANP, or the rat ANP precursor with a molecular weight of 13,000. A chromatographic profile by RP-HPLC is shown in Figure 2. α-rANP-LI with a low molecular weight in the spinal cord consisted of two major peaks eluted in the vicinity of the position of α-rANP. These two peaks co-migrated with synthetic α-rANP(4-28) and α-rANP(5-28), whereas no appreciable peak corresponding to α-rANP was observed.

DISCUSSION

ANP is one of bioactive peptides first isolated in peripheral organs and subsequently shown to exist in the brain (1-8,22,23). In this study, we have demonstrated that ANP is distributed throughout the spinal cord with the highest concentration in the sacral spinal cord. We previously reported the regional distribution of ANP in the rat brain. The highest concentrations of ANP (20-22 ng/g) were in the hypothalamus and the septum, followed by the midbrain (8 ng/g) (5). The ANP concentration in the sacral spinal cord was comparable to that in the midbrain. There are a few preliminary reports about ANP in the spinal cord (22,23). Using an immunohistochemical technique, Skofitsch et al. showed ANP immunoreactive fibers in the cervical and thoracic spinal cord in rats (22). Zamir et al. reported a low concentration of ANP in the cervical spinal cord by a RIA (23). The present study is the first to elucidate the entire distribution of ANP throughout the spinal cord.

Using the HP-GPC and RP-HPLC coupled with RIA, we previously showed that the major component of α-rANP-LI in the rat brain is a low molecular weight form (5,6) and that the predominant molecular forms with a low molecular weight are α-rANP(4-28) and α-rANP(5-28), whereas no α-rANP with 28 amino acids, a major circulating form, was detected in the brain (10). Thus, we proposed that ANP in neurons is generated by different post-translational processing from that in cardiocytes.

Since a paired basic amino acid residues, Arg^3-Arg^4, of α-rANP are known to be a typical processing signal (24), it is likely that the cleavage at these sites generates α-rANP(4-28) and α-rANP(5-28) in the central nervous system (10). On the other hand, a single basic amino acid, Arg, preceding the amino acid sequence of α-rANP in the rat ANP precursor is the processing site in the heart (2). The HP-GPC and RP-HPLC profiles of ANP in the spinal cord shown in this study are identical with those in the brain, confirming our previous hypothesis (10).

The role of ANP in the spinal cord is not clear at present. Interestingly, vasopressin, whose action on the renal function is opposite to that of ANP, is distributed in a similar pattern to ANP in the spinal cord with the highest concentration in the lumbo-sacral spinal cord (25). Intrathecal injection of vasopressin is reported to modulate the renal function via its action on the spinal cord (26). Therefore, ANP in the spinal cord might participate in the control of the renal function in a similar manner. In addition, since the sacral cord with the highest level of ANP is associated with the function of pelvic viscera, ANP in this region may be involved in the urogenital function.

The present study also showed that the level of ANP in the autonomic ganglion is comparable to that of the spinal cord. Recently, the presence of ANP in small intensely fluorescent cells in the paracervical ganglion (27) and coeliac-superior mesenteric ganglion was reported (28). In addition, ANP was found in rat adrenomedullary cells (28) and ANP-containing fibers were also stained in the dorsal vagal complex consisting of the solitary nucleus and vagal motor nucleus (7). These observations suggest that ANP regulates autonomic nervous function.

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REFERENCES

1. Flynn, T.G., de Bold, M.L., and de Bold, A.J. (1983) Biochem. Biophys. Res. Commun. 117, 859-865.
2. Kangawa, K., and Matsuo, H. (1984) Biochem. Biophys. Res. Commun. 118, 131-139.
3. Needleman, P., Adams, S.P., Cole, B.R., Currie, M.G., Geller, D.M., Michener, M.L., Saper, C.B., Schwartz, D., and Standaert, D.G. (1985) Hypertension 7, 469-482.
4. Cantin, M., and Genest, J. (1985) Endocri. Rev. 6, 107-127.
5. Morii, N., Nakao, K., Sugawara, A., Sakamoto, M., Suda, M., Shimokura, M., Kiso, Y., Kihara, M., Yamori, Y., and Imura H. (1985) Biochem. Biophys. Res. Commun. 127, 413-419.
6. Morii, N., Nakao, K., Kihara, M., Sakamoto, M., Sugawara, A., Shimokura, M., Kiso, Y., Yamori, Y., and Imura, H. (1986) Hypertension 8 suppl.I, I-61-65.
7. Kawata, M., Nakao, K., Morii, N., Kiso, Y., Yamashita, H., Imura, H., and Sano, Y. (1985) Neuroscience 16, 521-546.
8. Kawata, M., Ueda, S., Nakao, K., Morii, N., Kiso, Y., Imura, H., and Sano, Y. (1985) Histochemistry 83, 1-3.
9. Nakao, K., Morii, N., Itoh, H., Yamada, T., Shiono, S., Sugawara, A., Saito, Y., Mukoyama, M., Arai, H., Sakamoto, M., and Imura, H. (1987) J. Hypertension in press.
10. Shiono, S., Nakao, K., Morii, N., Yamada, T., Itoh, H., Sakamoto, M., Sugawara, A., Saito, Y, katsuura, G., and Imura, H. (1986) 135, 728-734.
11. Nakamura, M., Katsuura, G., Nakao, K., and Imura, H. (1985) Neurosci. Lett. 58, 1-6.
12. Itoh, H., Nakao, K., Katsuura, G., Morii, N., Shiono, S., Yamada, T., Sugawara, A., Saito, Y., Matsushita, A., and Imura, H. (1986) Neurosci. Lett. in press. 12. Katsuura, G., Nakamura, M., Inouye, K., Kono, M., Nakao, K., and Imura, H. (1986) Eur. J. Pharmacol. 121, 285-287.
13. Katsuura, G., Nakamura, M., Inouye, K., Kono, M., Nakao, K., and Imura, H. (1986) Eur. J. Pharmacol. 121, 285-287.
14. Itoh, H., Nakao, K., Morii, N., Sugawara, A., Yamada, T., Shiono, S., Saito, Y, Mukoyama, M., Arai, H, Sakamoto, M., and Imura, H. (1987) Jpn. Circ. J. in press.
15. Itoh, H., Nakao, K., Katsuura, G., Morii, N., Shiono, S., Sakamoto, M., Sugawara, A., Yamada, T., Saito, Y., Matsushita, A., and Imura, H. (1986) Circ. Res. 59, 342-347.
16. Itoh, H., Nakao, K., Morii, N., Yamada, T., Shiono, S., Sakamoto, M., Sugawara, A., Saito, Y., Katsuura, G., Shiomi, T., Eigyo, M., Matsushita, A., and Imura, H. (1986) Brain Res. Bull. 16, 745-749.
17. Nakao, K., Morii, N., Itoh, H., Sugawara, A., Sakamoto, M., Yamada, T., Shiono, S., Saito, Y., Katsuura, G., Nakamura, M., Eigyo, M., Matsushita, A., Kawata, M., Sano, Y., and Imura, H. (1986) in: Brain and blood pressure control (Nakamura, K., eds), pp195-204, Elsevier Science Publishers BV, Amsterdam.
18. Yamada, T., Nakao, K., Morii, N., Itoh, H., Shiono, S., Sakamoto, M., Sugawara, A., Saito, Y., Ohno, H., Kanai, A., Katsuura, G., Eigyo, M., Matsushita, A., and Imura, H. (1986) Eur. J. Pharmacol. 125, 453-456.

19. Itoh, H., Nakao, K., Katsuura, G., Morii, N., Yamada, T., Shiono, S., Sakamoto, M., Sugawara, A., Saito, Y., Eigyo, M., Matsushita, A., and Imura, H. (1986) Neurosci. Lett. 69, 254-258.
20. Nakao, K., Katsuura, G., Morii, N., Itoh, H., Shiono, S., Yamada, T., Sugawara, A., Sakamoto, M., Saito, Y., Eigyo, M., Matsushita, A., and Imura, H. (1986) Eur. J. Pharmacol. 131, 171-178.
21. 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.
22. Skofitsch, G., Jacobowitz, D.M., Eskay, R.L., and Zamir, N. (1985) Neuroscience 16, 917-948.
23. Zamir, N., Skofitsch, G., Eskay, R.L., and Jacobowitz, D.M. (1986) Brain Res. 365, 105-111.
24. Docherty, K., and Steiner, D.F. (1982) Annu. Rev. Physiol. 44, 625-638.
25. Millan, M.J., Millan, M.H., Czlonkowski, A., and Herz, A. (1984) Neuroscience 13, 179-187.
26. Riphagen, C.L., and Pittman, Q.J. (1985) Brain Res. 336, 346-349.
27. Papka, R.E., Trauring, H.H., and Wekstein, M. (1985) Neurosci. Lett. 61, 285-290.
28. Inagaki, S., Kubota, Y., Kito, S., Kangawa K., and Matsuo, H. (1986) Regul. Peptides 15, 249-260.