Central Dipsogenic Effect of Synthetic Rat Atrial Natriuretic Polypeptide in Normotensive Rats

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Accepted August 29, 1987

Abstract—The effect of intracerebroventricular (i.c.v.) injection of synthetic rat atrial natriuretic polypeptide (α-rANP) on drinking behavior was studied in normotensive rats. α-rANP (0.2, 0.4 or 0.8 μg in 5 μl) caused a dose-dependent dipsogenic effect which was abated by i.c.v. pretreatment with saralasin (9 μg in 5 μl). These results suggest that α-rANP possesses dipsogenic effects in water repleted rats and that brain angiotensin is involved. In addition, our data indicate that, at least as far as the effect of cerebral ANP is concerned, there are some differences between α-rANP and human atrial natriuretic polypeptide.

The saluretic, diuretic and hypotensive actions of atrial natriuretic factor (ANF) were first demonstrated by injection of homologous atrial extracts into anaesthetized rats (1). Later, extracts of atria from several vertebrates were shown to elicit the biological activities attributed to ANF (2, 3) even when tested in non-homologous species. The amino acid sequences of both human and rat ANFs were determined and synthetized (4). The synthetic rat ANF corresponds to a 26 amino acid sequence, while the human one is a 28 residue peptide and differs from the rat peptide mainly by the addition of Ser-Leu at the N-terminal and the substitution of methionine for isoleucine at position 110.

Although ANF was first found in rat and human atria, several evidences have suggested the presence of ANF in the hypothalamus and septum (5–7), regions mainly involved in central control of the cardiovascular apparatus and water intake. As a matter of fact, it has been demonstrated that i.c.v. injection of α-human atrial natriuretic polypeptide (α-hANP) antagonizes the action of angiotensin II (A II) on drinking behavior (8).

Recently, however, it has been reported that rat hypothalamus mainly releases a low molecular weight form of ANF (9). In light of the above findings, we studied the effect of α-rANP, which resembles the features of the ANF released from rat hypothalamus, on water intake in normotensive rats.

Male Wistar rats, weighing 275–300 g and aged 15 weeks, were used for these experiments. The animals were anaesthetized with chloral hydrate, and a permanent stainless steel cannula was implanted stereotaxically into a lateral ventricle, as previously reported (10). Four days were allowed for recovery before the animals were used. All the experiments were performed between 10:00 and 14:00 a.m. At the completion of the experiments, dye was injected in each animal through the cerebral ventricle to confirm the injection site.

Synthetic α-rANP (purchased from Merck Sharp and Dohme) and saralasin (purchased from Sigma) were dissolved in 0.9% NaCl solution (saline).

Each animal received only one dose of drug. The data are expressed as means±S.D. Statistical analysis was performed by using Student’s t-test: P<0.05 was considered significant.

As shown in Table 1, i.c.v. injection of α-
rANP (0.2, 0.4 or 0.8 μg in 5 μl) significantly stimulated drinking in water repleted rats. This effect was dose-dependent, and the maximum effect was achieved with the 0.8 μg dose.

I.c.v. pretreatment (2 min before α-rANP) with saralasine (9 μg in 5 μl) significantly reduced the dipsogenic effect induced by 0.8 μg of α-rANP (Table 1). In previous experiments, this dose of saralasine had been shown to block the dipsogenic effect induced by i.c.v. injection of angiotensin II (250 ng in 5 μl).

Our results indicate that the i.c.v. injection of synthetic α-rANP in normotensive rats causes a marked dipsogenic effect. This effect seems to be dose-dependent, and it is antagonized by an i.c.v. pretreatment with saralasine.

Previous findings have pointed out that i.c.v. injection of α-rANP significantly decreases drinking of water deprived rats, while i.c.v. injection of anti-ANF antiserum enhances water intake induced either by water deprivation or i.c.v. injection of angiotensin II (250 ng in 5 μl).

These findings, taken together, strongly support the idea that brain ANF is mainly involved in the control of water balance. It has been shown that brain and blood borne angiotensin II strongly increases in water deprivation (11).

More specifically, therefore, the above results would suggest that brain ANF can antagonize the action of angiotensin II on drinking behavior.

We used α-rANP and the results of our experiments are in contrast with the above mentioned results. As a matter of fact, at least under these experimental conditions, α-rANP produced a strong dipsogenic effect which was abated by pretreatment with i.c.v. saralasine, thus indicating an involvement of brain angiotensin.

This would suggest that, as far as the central effect of ANP is concerned, there may exist some species-specificity and that the effects induced by i.c.v. injections of ANP depend upon the peptide amino acid sequence.

In conclusion, whatever the mechanism, as far as we know, this is the first report indicating that rat α-rANP elicits dipsogenic activity when injected i.c.v. into normotensive animals.

Table 1. Effect of synthetic α-rat atrial natriuretic polypeptide on water intake

| Treatment                  | No. of rats | Water intake (ml/100 g b.w.) |
|----------------------------|-------------|-------------------------------|
|                            |             | 10  | 15  | 20  |
| Saline, 5 μl               | 6           | N.D.| N.D.| N.D.|
| α-rANP, 0.2 μg             | 6           | 1.4±0.289 | 1.7±0.209 | 2.3±0.196 | 2±0.196|
| α-rANP, 0.4 μg             | 6           | 1.5±0.147 | 2.3±0.216* | 3.2±0.098* | 3.4±0.219*|
| α-rANP, 0.8 μg             | 6           | 2.3±0.196**| 3.6±0.198**| 4.3±0.147**| 4.3±0.147**|
| Sar, 9 μg+Saline 5 μl     | 6           | N.D.| N.D. | N.D. |
| Sar, 9 μg+α-rANP 0.8 μg    | 6           | 0.4±0.403***| 0.5±0.209***| 0.6±0.403***| 0.7±0.298***|

The volume injected i.c.v. was always 5 μl. Values are the means±S.D. *P<0.001 vs. 0.2 μg of α-rANP, **P<0.001 vs. 0.4 μg of α-rANP. ***P<0.01 vs. 0.8 μg of α-rANP alone. Sar=Saralasine; Saralasine was injected i.c.v. 2 min prior α-rANP, N.D.=no discernible effect.

References
1 de Bold, A.J., Borenstein, H.B., Veress, A.T. and Sonnenberg, H.: A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. Life Sci. 28, 89–94 (1981)
2 Trippodo, N.C., MacPhee, A.A. and Cole, F.E.: Partially purified human and rat atrial natriuretic factor. Hypertension 5, Supp. I, I-81–I-88 (1983)
3 Kangawa, K., Fukuda, A., Kubota, I., Hayashi, Y. and Matsuo, H.: Identification in rat atrial tissue of multiple forms of natriuretic polypeptides of about 3,000 daltons. Biochem. Biophys. Res. Commun. 121, 585–591 (1984)
4 Nutt, R.F., Brady, S.F., Lyle, T.A., Dysonco, C., Paleveda, W.J., Ciccarone, T.M., Blaine,
E.H., Winquist, R.J., Bennett, C.D., Hirschmann, R. and Veber, D.F.: Synthesis of peptides with atrial natriuretic factor sequence. In Peptides: Proceedings of the 18th European Peptide Symposium, Djuronaset, Edited by Ragnarsson, U., p. 513–516, Almqvist & Wiksell Int., Stockholm (1984)

5 Morii, N., Nakao, K., Sugawara, A., Sakamoto, M., Suda M., Shimokura, M., Kiso, Y., Kihara, M., Yamori, Y. and Imura, H.: Occurrence of atrial natriuretic polypeptide in brain. Biochem. Biophys. Res. Commun. 127, 413–419 (1985)

6 Kawata, M., Nakao, K., Morii, N., Kiso, Y. Yamashita, H., Imura, H. and Sano, Y.: Atrial natriuretic polypeptide: Topographical distribution in the rat brain by radioimmunoassay and immunohistochemistry. Neuroscience 16, 521–546 (1985)

7 Tanaka, I., Misono, K.S. and Inagami, T.: Atrial natriuretic factor in rat hypothalamus, atria and plasma: determination by specific radioimmunoassay. Biochem. Biophys. Res. Commun. 124, 663–668 (1984)

8 Katsuura, G., Nakamura, M., Inouye, K., Kono, M., Nakao, K. and Imura, H.: Regulatory role of atrial natriuretic polypeptide in water drinking in rats. Eur. J. Pharmacol. 121, 285–287 (1986)

9 Tanaka, I. and Inagami, T.: Release of immunoreactive atrial natriuretic factor from rat hypothalamus in vitro. Eur. J. Pharmacol. 122, 353–355 (1986)

10 Squadrito, F., Trimarchi, G.R., Lupica, S., Magri, V., Costa, G., Brezenoff, H.E. and Caputi, A.P.: Cerebral cholinergic control of rat arterial blood pressure in streptozotocin-induced diabetes. Pharmacol. Res. Commun. 18, 951–965 (1986)

11 Phillips, M.I., Hoffman, W.E. and Bealer, S.L.: Dehydration and fluid balance: Central effects of angiotensin. Fed. Proc. 41, 2520–2527 (1982)