Vol. 119, No. 2, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 15, 1984 Pages 685-688

COMPARATIVE VASOACTIVE EFFECTS OF NATIVE AND SYNTHETIC ATRIAL NATRIURETIC FACTOR (ANF)

R. Garcia[1], G. Thibault, R.F. Nutt[2], M. Cantin and J. Genest

Clinical Research Institute of Montreal
110 Pine Avenue West, Montreal, Quebec, Canada H2W 1R7
[2] Merck Sharp & Dohme Research Laboratories, West Point, PA 19486

Received January 16, 1984

The presence of a natriuretic factor in mammals atria has been confirmed by many laboratories. In addition to its natriuretic and diuretic activities, atrial preparation of uncertain degree of purification are known to contain a vasoactive substance. By the use of pure atrial natriuretic factor of known amino acid sequence and its synthetic homologue we have presented strong evidences suggesting that natriuretic and vasoactive activities are indeed mediated by the same peptide. As this peptide has not yet been detected in the circulation, its actual physiological relevance remains to be elucidated.

The presence of a powerful natriuretic peptide(s) in atrial specific granule has received ample confirmation (1-4).

Several investigators have demonstrated that in addition to this natriuretic material, atrial extracts contain a vasoactive substance (5, 6); however, since crude atrial preparations were used, a clear relationship with ANF could not be established. Using a purified material, it has been suggested that both activities, natriuretic and vasoactive, are mediated by the same peptide (7).

Recently, ANF has been purified to homogeneity (8) and its amino acid sequence determined (9). The complete sequence of the longest peptide is composed of 33 amino acids and is referred to as ANF (1-33). Furthermore, a peptide with the amino acid sequence of native ANF was synthesized at the Merck Sharp & Dohme Research Laboratories (9), its natriuretic activity has been compared with native ANF of known amino acid sequence and has been found to be similar (9). The comparative vasoactive activity between both forms is reported here.

[1] To whom correspondence should be addressed.

0006-291X/84 $1.50

METHODS

Vascular strips

Male New Zealand rabbits (1.8 - 2.0 kg) were anesthetized with sodium pentobarbital (30 mg/kg i.v.), the renal arteries were rapidly excised, the excess fat and connective tissue were gently trimmed off and the arterial tissue was helically cut. Each vascular strip, 1 mm by 15-20 mm, was suspended in a 20-ml tissue bath containing a continuously oxygenated (95% O_2 - 5% CO_2) Krebs solution at 37°C and pH 7.4. The strips were mounted between a fixed base and a force displacement transducer (Grass, FT-03C). The contractions were registered on a model 7 Grass polygraph.

A tension of 500-700 mg was applied to each strip. This tension was adjusted and bathing fluid changed every 15 min. The strips were allowed to equilibrate for 2 hours before the experimental procedure began.

The composition of the solution used in this study was (mmol/liter): NaCl 119; KCl, 4.7; KH_2PO_4, 1.8; $MgSO_4 \cdot 7H_2O$, 1.17; $CaCl_2 \cdot 6H_2O$, 2.5; $NaHCO_3$, 25.0; and dextrose, 5.5.

For each arterial strip a cumulative dose-response curve to norepinephrine (NE) (L-Norepinephrine bitartrate, Sigma Chemicals) was constructed. Once the standard curve was reproducible, it was repeated 5 minutes after adding into the bath the following peptides: native ANF 3-33 (2.3 nM, $n = 4$), synthetic ANF 8-33 acid (2.53 nM, $n = 5$), and synthetic ANF 8-33 amide (4.16 nM, $n = 5$). The contraction elicited for each dose of NE is expressed as a percent of the maximum response.

Results are expressed as means ± SEM. Analysis of covariance and the Dunnet test were used to compare multiple dose-response curves.

RESULTS

Figure 1 shows that both, synthetic and native ANF displace the NE dose-response curve to the right ($P < 0.01$). This effect was more remarkable at lower doses, where the threshold to NE was raised in the presence of ANF. No difference was seen between native and synthetic ANF.

DISCUSSION

A vasoactive substance has been found in crude atrial preparation (5, 6) without a clear relationship with ANF. Using a more purified ANF it was later shown (7) that ANF itself produces a potent dose-dependent relaxant effect on vascular smooth muscle strips contracted by either NE or angiotensin II and a vasodilator effect in the isolated perfused rat kidney. In the present work, by using pure native rat ANF with known amino acid sequence and known natriuretic activity (8, 9), and its synthetic homologue, we have given strong evidence suggesting that natriuresis and vasoactivity are mediated by the same peptide.

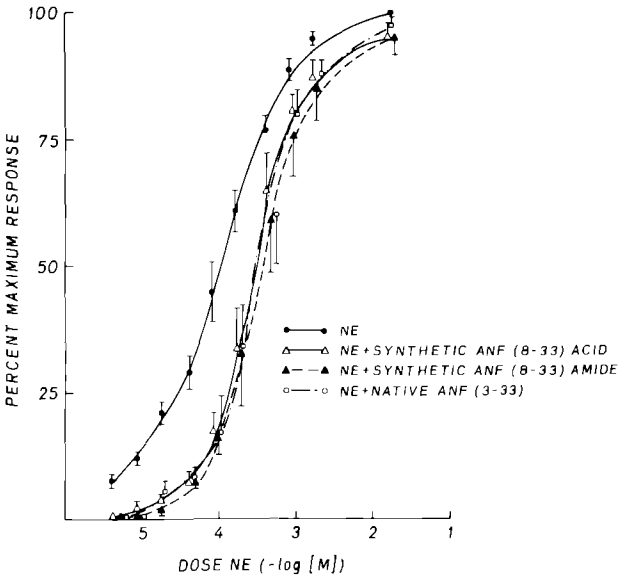

Figure 1: Comparative vasoactive effect between native and synthetic ANF.

The mechanism(s) of the effect of ANF on natriuresis and vascular smooth muscle is not well known. It seems that an inhibition of the Na-K ATPase is not involved (4). *In vivo*, the injection of ANF increases urinary excretion and plasma levels of cGMP. *In vitro*, incubation of ANF with rat minced kidney produces an increase in tissue levels of cGMP and a decrease in cGMP phosphodiesterase (10). Nitroprusside mimicks the effect of ANF in smooth muscle (7), suggesting again an involvement of cGMP.

It is well established that atrial receptors play an important role in water and sodium excretion (11). The presence in the mammal atria of this peptide with powerful natriuretic and vasodilatory effects, suggests that it could be involved in the regulation of blood volume and vascular resistance. However, since the release of ANF into the circulation has not yet been demonstrated its actual physiological relevance remains to be elucidated.

REFERENCES

1. de Bold, A.J. (1982) Can. J. Physiol. Pharmacol. 60, 324-330.
2. Garcia, R., Cantin, M., Thibault, G., Ong, H., and Genest, J. (1982) Experientia 38, 1071-1073.
3. de Bold, A.J., and Flynn, T.G. (1983) Life Sci. 33, 292-302.
4. Thibault, G., Garcia, R., Cantin, M., and Genest, J. (1983) Hypertension 5 (Suppl. I), I-75-I-80.

5. Keth, R.C., Hong, K., Fukazawa, S., Rocco, R., Sewart, J.L., Lynch, C.J., and Wawad, R. (1982) Fed. Proc. 41, 983a.
6. Currie, M.G., Geller, D.M., Cole, B.R., Boylan, J.G., Yu Sheng, W., Hollenberg, S.W., and Needleman, P. (1983) Science 221, 71-73.
7. Garcia, R., Thibault, G., and Genest, J. (1983) Clin. Invest. Med. 6 (Suppl. 2), 56a.
8. Thibault, G., Garcia, R., Seidah, N.G., Lazure, C., Cantin, M., Chrétien, M., and Genest, J. (1984) FEBS Lett., in press.
9. Seidah, N.G., Lazure, C., Chrétien, M., Thibault, G., Garcia, R., Cantin, M., Genest, J., Nutt, R.F., Brady, S.F., Lyle, T.A., Paleveda, W.J., Colton, C.D., Ciccarone, T.M., and Veber, D.F. (1984) Proc. Natl. Acad. Sci., in press.
10. Hamet, P., Thibault, G., Tremblay, J., Garcia, R., Gutkowska, J., Cantin, M., and Genest, J. Submitted to Science.
11. Gauer, O.H., Henry, J.P., and Belm, C. (1970) Ann. Rev. Physiol. 32, 547.