The Heart as an Endocrine Gland

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SUMMARY The sequence of atrial natriuretic factor (ANF) has been determined, as well as the complete structure of the rat and human complementary DNA and gene. ANF and ANF messenger RNA are present not only in atria but also in ventricles. The circulating form of ANF has been identified as the C-terminal of the molecule, ANF (Ser 99-Tyr 126). The isolated secretory granules of rat atrial cardiocytes contain only pro-ANF (Asn 1-Tyr 126). An enzyme (IRCM-SP1) has been isolated from heart atria and ventricles. This enzyme is highly specific in cleaving ANF (Asn 1-Tyr 126), to yield ANF (103-126), (102-126), and (99-126). In target cells, ANF produces a rise in cyclic guanosine 3',5'-monophosphate (cGMP) due to activation of particulate guanylate cyclase, and inhibition of adenylate cyclase leading in some cases to a decrease in cyclic adenosine 3',5'-monophosphate (cAMP). ANF produces relaxation of rabbit and rat aortic strips, inhibits steroidogenesis in both zona glomerulosa and zona fasciculata cells, and inhibits the release of arginine vasopressin from the isolated rat hypothalamic-hypophysectomy preparation in vitro but decreases AVP release in vivo only at pharmacological doses. In all forms of experimental hypertension, plasma levels of ANF are increased and, at some time periods, atrial levels are also decreased. The ventricular levels of immunoreactive ANF are also increased in renal hypertension. Infusion of ANF by minipumps decreases the blood pressure near control levels in several models of experimental hypertension. In cardiomyopathic hamsters with heart failure, the atrial levels of immunoreactive ANF are decreased while the plasma and ventricular levels are increased. In humans, the mean plasma levels of ANF are not increased above control values in essential hypertension (for moderate increases in blood pressure), but they are increased in the aortic blood in renal hypertension. Plasma levels of immunoreactive ANF are increased in paroxysmal tachycardia, valvular heart diseases, idiopathic cardiomyopathies, and coronary heart disease. (Hypertension 10 [Suppl I]: 1-118—1-121, 1987)

KEY WORDS atrial natriuretic factor • molecular biology • biochemistry • physiology • physiopathology

It is now well established that the atria are endocrine glands secreting, in rats and humans, the 28-amino acid peptide atrial natriuretic factor (ANF)-(Ser 99-Tyr 126). ANF messenger RNA (mRNA) and immunoreactive ANF (irANF) are also present, although in much lower amounts, in ventricular cardiocytes.

Biochemistry and Molecular Biology of ANF

Isolation and Sequencing

After the discovery by de Bold et al. that rat atrial extracts produce diuresis and natriuresis, we showed that the effects of the crude extracts are localized in the specific (now secretory) granules themselves. Early purification attempts established the polypeptide nature of ANF and the amino acid composition of short (C-terminal) peptides. In June 1983 we isolated and sequenced, among others, a 33-amino acid peptide that is part of a larger molecule containing at least 106 amino acids. After its sequencing in our laboratory, the 33-amino acid peptide was synthesized by Merck Sharp & Dohme in September 1983. All our further work reported here has been done with this synthetic peptide. The C-terminal of human ANF has also been isolated and sequenced.

Cloning the Complementary DNA and Gene of ANF

The availability of atrial peptide sequence data prompted cloning of the complementary DNA (cDNA) and then of the gene for both rat and human ANF. Rat cDNA has been cloned. The rat gene has also been isolated and its structure determined. Human cDNA for ANF and the human gene have also been delineated.
Chromosomal Localization of the ANF Gene

Chromosomal assignment of the gene coding for human ANF was accomplished by in situ hybridization of a [3H]ANF probe to a human chromosome preparation and Southern blot analysis of somatic cell hybrid DNA with normal and rearranged chromosomes. The human ANF gene was mapped to the distal short arm of chromosome 1 in band 1P36. Southern blot analysis of hybrid mouse × Chinese hamster somatic cells was used to assign the mouse ANF gene to chromosome 4.11

Localization of Immunoreactive ANF in the Heart

Up to now, it has been well established that the atria are endocrine glands. Recent studies indicate that ventricular cardiocytes also harbor immunoreactive ANF (irANF) and contain and secrete it in the medium when cultured.12 Biochemical studies show that the irANF present in ventricular cardiocytes is, as in the atria, of the high-molecular-weight variety.12 Cardiac ventricular cardiocytes of control animals contain ANF messenger RNA (mRNA).13

Circulating Form of ANF

The circulating form of ANF has been isolated from plasma of morphine-treated rats as the C-terminal, active portion of the molecule, ANF (Ser 99–Tyr 126).14, 15

Bio synthesis

Secretory granules from rat atria were isolated by differential centrifugation and by a 53% Percoll gradient. Analysis of the ANF content in these isolated granules by high performance liquid chromatography (HPLC), amino acid analysis, and sequencing demonstrated that it was made up only of pro-ANF (Asn 1–Tyr 126). The precursor was present in all granules as demonstrated by immunocytochemistry using antibodies against synthetic N-terminal fragments of the propeptide.16 An enzyme (IRCM-SP1) has been isolated from rat heart atria and ventricles. Rat IRCM-SP1 was shown to be highly specific in cleaving ANF (Asn 1–Tyr 126) to yield ANF (103–126), (102–126), and (99–126) in that order of preference. The enzyme was nine times more abundant in atria than in ventricles per milligrams protein.17

ANF released by the isolated perfused heart (Langendorff preparation) was extracted from the perfusate by C$_n$ Sep-Pak cartridges and then isolated by immunoaffinity chromatography and reverse phase HPLC. About 500 µg of irANF was thus obtained and submitted to amino acid sequencing. The C-terminal Tyr was detected by radiolabeling. Identification of these residues indicated that the primary structure corresponds to ANF (Ser 99–Tyr 126).18 Injection of $^{125}$I-ANF (Glu 54–Tyr 126) in the perfusion fluid and analysis of the perfused material revealed no change of this relatively large peptide.18 When $^{125}$I-ANF (Asn 1–Tyr 126) is incubated in whole blood, plasma, or serum for different time intervals, the results indicate minimal cleavage of the propeptide in whole blood or plasma. Incubation with serum results in the formation of 11.7-kDa and 2.7-kDa peptides, which correspond to the N- and C-terminal portions of the molecule respectively. These results suggest that hydrolysis of the propeptide in serum is mediated by an enzyme activated during coagulation and thus has no physiological relevance.

Atrial cardiocytes in culture contain and secrete large amounts of irANF in the medium.19 The irANF present in these cells is exclusively of the high M, variety, as is most of what is secreted, with only about 15 to 25% being secreted as the low M, form.

Second Messengers

In all target cells so far investigated, ANF produces a rise in cyclic guanosine 3′,5′-monophosphate (cGMP) modulated by activation of particulate guanylyl cyclase,20, 21 and an inhibition of adenylate cyclase leading in some cases to a decrease in cyclic adenosine 3′,5′-monophosphate (cAMP).22, 23

Physiology

Kidneys

Receptors have been localized by radioligand-binding studies and by radioautography in the podocytes of visceral epithelial cells of glomeruli, the vasa recta bundles of the outer medulla, and the collecting duct cells of the inner medulla24, 25; binding is present in glomeruli, thick ascending limbs of Henle, and collecting duct membranes, but not in proximal tubules.26 In glomeruli, a 50-fold increase of cGMP over basal levels occurs after exposure to ANF, while a two-fold increase occurs in the ascending limbs, and a three-fold increase in collecting duct cells.21 ANF inhibits adenylate cyclase activity in glomeruli, collecting ducts, and loops of Henle, but not in proximal tubules.27 No metabolic change occurs in any isolated nephron segment after exposure to ANF.28

Blood Vessels

ANF produces a potent, dose-dependent, relaxing effect on rabbit and rat aortic strips.29, 30

Adrenal Cortex

ANF inhibits steroidogenesis prior to progesterone synthesis in both zona glomerulosa and zona fasciculata of the adrenal cortex.31–33

Arginine Vasopressin Release

ANF inhibits the release of arginine vasopressin from the isolated rat hypothalamohypophysial preparation in vitro34 but does so in vivo only at pharmacological levels.35

Physiopathological Implications

Animals

Hypertension

In all forms of experimental hypertension, plasma levels of ANF are increased and, at some time periods,
atrial levels are decreased.36–38 The ventricular levels of irANF are also increased in renal hypertension, and typical secretory granules (as delineated by ultrastructural immunocytochemistry using an immunogold technique) appear in ventricular cardiocytes. Infusion of ANF (Arg 101–Tyr 126) with minipumps at 100 ng/hr for several days brings down the blood pressure of hypertensive animals to control levels in several models.39–41 In the two-kidney, one clip model, only the saralasin-sensitive animals respond to ANF infusion by lowering of blood pressure.42

**Congestive Heart Failure**

In cardiomyopathic hamsters, atrial levels of irANF are decreased while the plasma and ventricular levels are increased.43 ANF mRNA, which is present in moderate amounts in the ventricular cardiocytes of controls, is markedly increased in congestive heart failure. Typical secretory granules harboring irANF are present in the ventricular cardiocytes of hamsters with heart failure while they are absent in control animals.43

**Humans**

**Hypertension**

The mean plasma levels of ANF are not increased above control values in essential hypertension (for moderate increases in blood pressure), but they are increased in aortic blood in renal hypertension.

**Cardiac Diseases**

Plasma levels of irANF are increased in paroxysmal tachycardia, valvular heart diseases, idiopathic cardiomyopathies, and coronary heart diseases.

It is now well established that the atria and possibly the ventricles as well are endocrine glands that secrete in the circulation a 28–amino acid peptide with far-ranging effects on the control of blood pressure and blood and extracellular fluid volume. The biosynthetic pathways of ANF in the heart, the factors controlling its release, and its physiological effects (as opposed to its pharmacological effects) remain to be determined. The renal effects of ANF are still not completely understood. The central nervous system effect of ANF as regards control of blood pressure and extracellular fluid volume still remains to be elucidated. The full clinical effects of ANF remain largely unknown. It is nevertheless almost certain that it will become a therapeutic agent in hypertension and in the clinical control of edematous states.

**Acknowledgments**

The work done on ANF at the Clinical Research Institute of Montreal has been from the start a collaborative effort primarily involving the members of the Multidisciplinary Research Group on Hypertension (Jacques Genest, Marc Cantin, Gaetan Thibault, Raoul Garcia, Jolanta Gutkowska, Pavel Hamet, André De Léan, Ernesto Schiffrin, Otto Kuchel, Madhu B. Anand-Srivastava, Johanne Tremblay, Stephen Pang), members of the Laboratory of Molecular Endocrinology (Michel Chrétien, Nabil G. Seidah, Claude Lazure), members of the Laboratory of Molecular Biology (Jacques Drouin, Mona Nemer), and Peter Schiller, and Ross Milne.

**References**

23. Anand-Srivastava MB, Srivastava AK, Cantin M. Pertussis
toxin attenuates atrial natriuretic factor-mediated inhibition of
adenylate cyclase: involvement of Gi-guanine nucleotide regulatory
Cantin M. Distinct localization of atrial natriuretic factor and
angiotensin II in the glomerulus. Am J Physiol 1986;251:
F594–F602
25. Bianchi C, Gutkowska J, Bullak J, Charbonneau C, Genest J,
Cantin M. Localization of 125I-atrial natriuretic factor (ANF)
binding sites in rat renal medulla. Light and electron micro-
scope radioautographic studies. J Histochem Cytochem 1987;
35:149–153
26. De Léan A, Vinay P, Cantin M. Distribution of atrial natriuret-
27. Anand-Srivastava MB, Vinay P, Genest J, Cantin M. Effect of
atrial natriuretic factor on adenylate cyclase in various nephron
ANF and various diuretics on isolated nephron segments of
atrial natriuretic factor on rat and rabbit vascular strips and
30. Schiffrin EL, Chartier L, Thibault G, St-Louis J, Cantin M,
Genest J. Vascular and adrenal receptors for atrial natriuretic
31. Chartier L, Schiffrin EL, Thibault G, Garcia R. Atrial natri-
uretic factor inhibits the effect of angiotensin II, ACTH and
potassium on aldosterone secretion in vitro and angiotensin II-
induced steroidogenesis in vivo. Endocrinology 1984;115:
2026–2028
32. De Léan A, Racz K, Gutkowska J, Nguyen TT, Cantin M,
Genest J. Specific receptor-mediated inhibition of synthetic
atrial natriuretic factor of hormone-stimulated steroidogenesis
in cultured bovine atrial cells. Endocrinology 1984;115:1636–
1638
33. Racz K, Kuchel O, Cantin M, De Léan A. Atrial natriuretic
factor inhibits the early pathway of steroid biosynthesis in
Cantin M. Effect of synthetic atrial natriuretic factor on argi-
nine vasopressin release by the rat hypothalamo-neurohypophy-
seal complex in organ culture. Biochem Biophys Res Com-
mun 1986;134:652–658
35. Januszewicz P, Larose P, Ong H, Gutkowska J, Genest J,
Cantin M. Effect of atrial natriuretic factor on plasma vasopressin
factor in spontaneously hypertensive rats. Hypertension 1986;
8(suppl I):1-137–140
37. Gutkowska J, Kuchel O, Racz K, Buu NT, Cantin M, Genest
J. Increased plasma immunoreactive atrial natriuretic factor
concentrations in salt sensitive Dahl rats. Biochem Biophys
38. Garcia R, Cantin M, Gutkowska J, Thibault G. Atrial natri-
uretic factor during development and reversal of one-kidney,
one clip hypertension. Hypertension 1987;9:144–149
low doses of atrial natriuretic factor (ANF Arg 101–Tyr 126) reduces blood pressure in conscious SHR without apparent
179:396–401
Reduction of blood pressure and increased diuresis and natri-
uresis during chronic infusion of atrial natriuretic factor (ANF
41. Garcia R, Thibault G, Gutkowska J, Cantin M. Effect of
chronic infusion of atrial natriuretic factor on plasma and ur-
inary aldosterone, plasma renin activity, blood pressure and
sodium excretion in 2K1C hypertensive rats. Clin Exp Hyper-
tens 1986;A8:1127–1147
42. Garcia R, Gutkowska J, Cantin M, Thibault G. Renin depen-
dency of the effect of chronically administered atrial natriuretic
factor in two-kidney, one clip rat. Hypertension 1987;9:88–95
atrial natriuretic factor (ANF) in experimental congestive heart
The heart as an endocrine gland.
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Hypertension. 1987;10:I118
doi: 10.1161/01.HYP.10.5_Pt_2.I118
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/10/5_Pt_2/I118

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