African Journal of Biotechnology Vol. 5 (25), pp. 2534-2539, 29 December, 2006
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2006 Academic Journals

Review

Atrial natriuretic peptide (ANP)-granules: ultrastructure, morphometry and function

Eliane Florencio Gama and Romeu Rodrigues de Souza*

Universidade São Judas Tadeu, Departamento de Anatomia Humana, São Paulo, Brasil.

Accepted 6 December, 2006

The atrial granules containing the peptide hormone, Atrial natriuretic peptide (ANP) are present in the four regions of the atrial-auricular complex (two atria and two auricles). ANP-immunoreactivity was detected in all granules from the four regions. Ultrastructurally, atrial myocytes show the presence of very electron dense granules, with sparsely granular and homogeneous content, coated with a double membrane. The number of granules is greatest in the right atrium followed by the left atrium and left auricle and right auricle, in this order. The diameter of granules in the cardiocytes is significantly largest in the right atrium and reduced via the left auricle to the left atrium and right auricle. These data lead to suppose that the right atrium is the one that most synthesizes and stores the ANP. The number of ANP-granules is influenced by several physiological conditions: temperature, dehydration and nutritional condition. The main physiological stimulus for increased ANP release is the atrial muscle stretch, which normally occurs when extra cellular fluid volume or blood volume is elevated. The ANP is eliminated through the atrial myocytes, via exocytosis. Granule content is released into the extra-cellular space (extrusion). The ANP causes diuresis, natriuresis, vasodilatation and depression of blood pressure. It is also involved in the modification of the water-electrolyte balance.

Key words: ANP-granules, ultrastructure, morphometry.

INTRODUCTION

Previous studies from the fifties (Kisch, 1956) in guinea pig hearts have shown the presence of specific atrial granules which has been functionally considered as an activator of sodium and water excretion and, consequently, blood pressure reduction (De Bold et al., 1981; Forssmann et al., 1984; Skepper and Navaratnam, 1988; Jiao et al., 1993; Yoshihara et al., 1998). Those granules, in the myocites of the auricles and in the atria, contain a peptide hormone called atrial natriuretic peptide (ANP). The atrial and auricle walls distension under conditions of hypervolemia or blood pressure increase would promote an increase in the circulating ANF (Varess and Sonnerberg, 1984). Thus far, five molecules comprise the natriuretic peptide family: Atrial natriuretic peptide (ANP), Urodilatin, Brain natriuretic peptide (BNP), C-type natriuretic peptide (CNP) and Dendroaspis natriuretic peptide (DNP) (Cea, 2005). Here, the ANP will be considered.

ULTRASTRUCTURE OF THE ANP-GRANULES

The electron micrographs of the atrial myocytes show the presence of very dense electron granules, with sparsely granular and homogeneous content, coated with a double membrane. Among those there are others less dense, but in a smaller quantity. Most of their granules are located near the nuclear poles, among mitochondria, golgi complex cisterns and rough endoplasmic reticulum, microtubules and myofilaments. In addition to the great number of clear vesicles near the perinuclear region, there is a small number near the plasma membrane (Figures 1 and 2).

*Corresponding Authors E-mail: souzarrd@uol.com.br. Tel: 551138899196

DISTRIBUTION OF THE ANP-GRANULES

Using immunocytochemistry, it is possible to detect with certainty the presence of ANP in the granules (Cantin, 1985). In the guinea pig heart ANP-immunoreactivity was detected in all granules, from all cardiocytes in the four regions of the atrial-auricular complex. Great amount of gold particles related to the granules has been observed, as well as less electron dense material near to them (Figures 3 and 4) (Gama et al., in press). In many species, including the guinea pig, the ANP-granules are also present in the ventricles (Mifune et al., 1992). It was also verified in dogs that the removal of the auricle bilaterally eliminates the ANP release (Stewart et al., 1992).

QUANTITATIVE STUDIES

Quantitative studies on the granules were performed in various mammalian species (Jamieson and Palade, 1964; Cantin et al., 1979; Mifune et al., 1996), but authors do not agree on the number and diameter of the granules in the various regions of the atrial-auricular complex. In the rat, the larger number of granules was found in the right atrium, whereas in the guinea pig, in the hamster, in the rabbit and in the cat, the number of granules was larger in the left atrium (Cantin et al., 1979). Chapeau et al. (1985) assert that there are a larger number of granules in the right atrium. According to Mifune et al. (1992), imunohistochemically, the most intensely peptide-reactive cardiocytes were localized in the right auricle.

In a recent study, Gama et al. (in press) observed that, although not significant, the number of granules in the right atrium was larger than in the other regions of the atrial-auricular complex. These results are in accordance with those of Rinne et al. (1986) and Chiu et al. (1994) to whom the right atrium is the greater source of ANP, not for presenting the peptide in a greater amount, but for responding with greater intensity to mechanical and nervous stimulus. Gama et al. (in press) observed that the amount of ANP-granules decrease from the right to the left auricle and from the right to the left atrium However, a greater amount of granules in the left atrium in relation to the right one has been reported in guinea pig hearts (Cantin et al., 1984). The results obtained by Skepper et al. (1989) and Avramovitch et al. (1995) indicate that there is no statistical difference from one side (right) in relation to the other (left) regarding the amount of granules in the atrial–auricular complex. Results showing that the right auricle is functionally more active than the left one have been reported (Varess and Sonnenberg, 1984; Skepper et al., 1989; Stewart et al., 1992). It was also verified in dogs that the removal of the auricle bilaterally eliminates the ANP release (Stewart et al., 1992). It is possible, that these different results are due to different methods used.

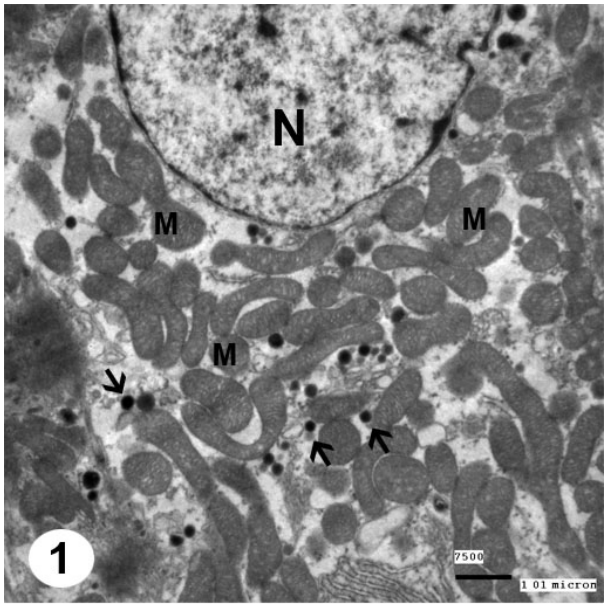

Figure 1. Electron micrograph of right atrium cardiocyte of the guinea pig heart. The ANP-granules (arrows) are found at the pole of the nucleus (N), among numerous mitochondria (M).

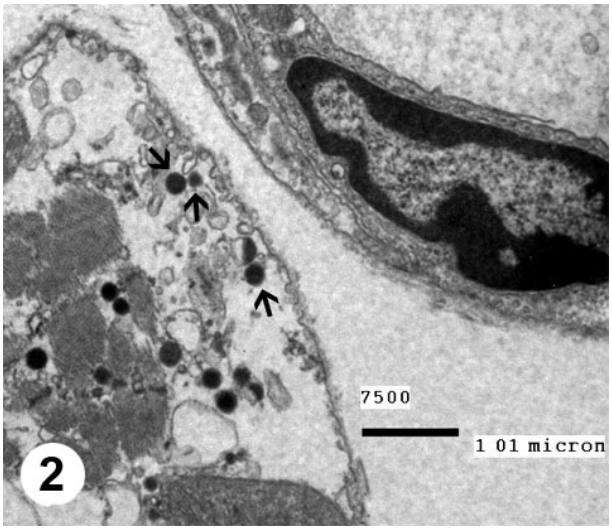

Figure 2. Electron micrograph from right auricle of guinea pig heart showing a number of granules (arrows) near the plasma membrane (P).

The number of ANP-granules is influenced by several physiological conditions: temperature, dehydration and nutritional condition (Mifune et al., 1993; Gall et al., 1990; Toyoshima et al., 1996; Penner et al., 1990; Nakayama et al., 1984; Takayanagi et al., 1985). A reduction of temperature from 37 to 27 degrees C caused a decrease of ANP release by 64% in the rat heart. The number of granules in the cardiocyte increased during dehydration

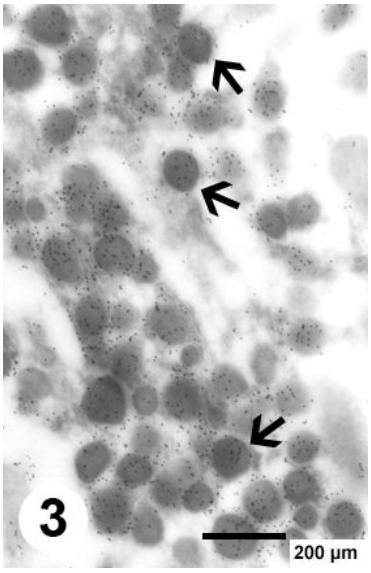

Figure 3. Immunocytochemical staining of ANP-granules in the right atrium (arrows). No immunocytochemical reaction was observed outside the granules. Observe the presence of numerous Protein-A Gold (10 nm), particles inside the granules.

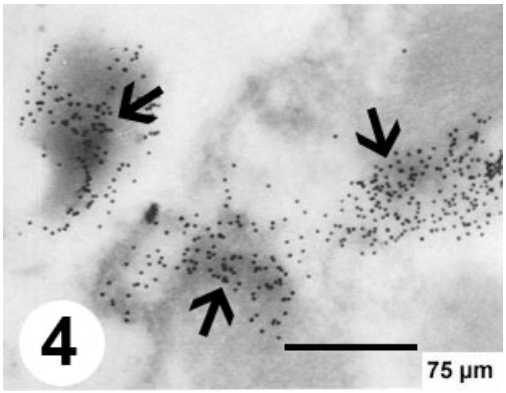

Figure 4. Immunocytochemical staining of ANP-granules in the right auricle. Several Protein – A Gold particles can be seen inside the granules (arrows).

(Gall et al., 1990; Toyoshima et al., 1996), but decreased after Na loading (Penner et al., 1990). Conversely, both the plasma ANP and ANP mRNA levels increased after loading (Nakayama et al., 1984; Takayanagi et al., 1985). These findings suggest that numerical changes in ANP-granules are closely associated with their synthesis and secretion in the cardiocytes, and that the synthetic and secretory ability is enhanced in the cell with fewer granules (Mifune et al., 1996), i. e., in the left atrium and auricles. Relatively low production of the ANP and/or a rapid secretion, with the consequent lower storage of the peptide in these regions, may be the reason.

It was demonstrated that there were significant differences in granule diameter among the regions of the atrial-auricular complex. The average diameter of the granules in the right atrium was larger than in the other regions. The distribution of the granules diameter from the right atrium was displaced towards larger values compared to data from the left atrium and auricles. According to the observations of Mifune et al. (1996), in various mammalian species the fewer number of granules the cardiocyte had, the smaller the granule size became, suggesting that the number of granules is possibly related to the granule size. The granule size is therefore possibly determined by the number of granules in the cardiocytes in the species (Mifune et al., 1996). The ratio of peptide content in the right atrium was larger than in the auricles and may indicate a more important endocrine role in right atrium than in left atrium and auricles.

The size of ANP-granules is also influenced by various factors. It was small in the presence of water depletion (Gall et al., 1990). ANP-granules become smaller during rapid synthesis, especially in spontaneously hypertensive rats (Takayanagi et al., 1985). It was reported that the enhancement of ANP synthesis in the cells reduced the granule size, or ANP-granules became smaller with a concomitant decrease in the levels of atrial ANP mRNA and plasma ANP in the course of the down regulation (Mifune et al., 1991, 1992) According to these authors, these findings suggest no relationship between the granule size and the ability of ANP synthesis and secretion.

ANP SECRETION

Since the discovery of ANP more than 20 years ago, numerous studies have focused on the mechanisms regulating ANP secretion. Specific atrial granules closeness to the sarcolema, the granule membrane fusion to the sarcolema and the presence of secretion-like amorphous material present in the endomysium and in the sub-endocardial space suggest an exocytosis (Figure 5) process (Gilloteaux et al., 1991). These observations complement previous descriptions (Imada et al., 1988; Needleman et al., 1989), demonstrating that the ANP is eliminated through the atrial myocytes, via exocytosis. Those authors suggested that the ANP would be released by the emiocytosis process, in which after diffusion through the sub-endocardial and sub-epicardial spaces would then be transported through the endocardium, epicardium and endothelium from vessels to the blood by means of an endocytosis mechanism, mediated by receptors. They also suggest that the atrium endothelial layer can play an important role in the transport control, in the pro-hormone activation and in the ANP release into blood stream. It was observed in the rat, that endothelin-1, a potent vasoconstrictor produced by endothelial cells, stimulates the secretion of the ANP by a direct mechani-

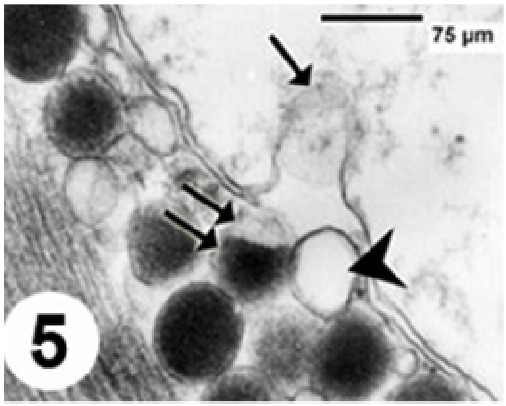

Figure 5. Electron micrograph of an atrial cardiocyte. Observe an empty granule (arrowhead) and a partially empty one (double arrow). Amorphous material, apparently derived from the granules, can be observed in the extra-cellular space (arrow).

sm not through a hemodynamic change (Horio et al., 1993). The dramatic increase in ANP release produced by cardiac ischemia appears to be mediated in part by endothelin. Nitric oxide, an important vasodilator, is also produced by endothelial cells and inhibits ANP secretion through cyclic GMP as an intracellular messenger (Dietz, 2005).

ANP FUNCTION

ANP is a peptide hormone that causes diuresis, natriuresis, vasodilatation, reduction of plasma renin-aldosterone concentration and depression of blood pressure (De Bold et al., 1981; Cantin and Genest, 1985; Toyoshima et al., 1996; Mifune et al., 2004; Cea, 2005). An anti-inflammatory ANP potential and a pro-apoptotic action in rat endothelial cells have also been described (Klinger et al., 2005). Studies carried out in humans (Tanaka et al., 1986) and in rabbits (Cho et al., 1991), assessed the atrial pressure, atrial wall dilatation and contraction frequency, and suggested that the major stimulus for ANP release is the frequency increase of cardiomyocytes shortening. However, an increase of atrial contraction frequency from 300 to 500/min, in the rat, did not cause a significant change in the ANP release (Katoh et al., 1990). According to Katoh et al. (1990), Schiebinger and Greening (1992), Seul et al. (1992); De Bold et al. (2001) and Dietz (2005) the main physiological stimulus for increased ANP release is the atrial muscle stretch, which normally occurs when extra cellular fluid volume or blood volume is elevated. It was showed (Katoh et al., 1990) that an elevation of left atrial filling pressure of working hearts from 8 to 18 and 28 cm H_2O was associated with pressure dependent, and reversible increase of the ANP release.

The morphofunctional findings with respect to the natriuretic peptides from the atria and auricles are becoming very useful, since an increasing concern has been observed in studies related to cardiac surgeries (Cox et al., 1991; McCarthy et al., 1993; Yoshihara et al., 1998) regarding the acute and chronic effects of the ANP release reduction and consequently abnormalities in the renal function related to body fluid control arising from cardiac surgeries where removal of the auricles is carried out. Furthermore, a number of clinical trials suggest that application of these peptides may represent a new pharmacological tool in the treatment or prevention of diseases like acute renal failure or cardiac remodeling in acute myocardial infarction and congestive heart failure (De Bold and De Bold, 2005; Michels and Tarnow, 2001). Elevated ANP and BNP levels may serve as an early warning system to help to identity patients at high risk for cardiac events. The clinical applications of natriuretic peptides are expanding rapidly. Recent basic science and clinical research findings continue to improve our understanding of this peptide system and guide use of ANP and BNP as biomarkers and as therapeutic agents (Silver, 2006).

CONCLUSIONS

In conclusion, the ANP is present in all the granules from the four regions of the atria and auricles and the right atrium is the region with the largest number of granules. The small granules are, by far, the most numerous in the four regions. It is possible that the wall of the right atrium is the most sensitive region to principal mechanism for the liberation of the ANP granules that is the distension of the wall musculature (De Bold and De Bold, 2005), because it is the area where the largest number of granules and those with the biggest size were concentrated. The significantly greater number of right atrial ANP positive granules suggest a greater volume capacity for the right atrial ANP- positive granules as compared to the other regions of the heart. This may indicate that ANP is secreted primarily from the right atrium and to a lesser extent from the left atrium and auricles and that both atrial and auricular ANP are closely related in chemical nature and immunocytochemical characteristics.

ACKNOWLEDGEMENT

RRS is career investigator of CNPq (National Council of Research).

REFERENCES

Avramovitch N, Hoffman A, Winaver J, Haramati A, Lewinson D (1995). Morphometric analysis of atrial natriuteric peptide-containing granules

in atriocytes of rats with experimental congestive heart failure. Cell Tissue Res 279: 575-583.
Cantin M, Timm-Kenedy M, El-Khatib E, Huet M, Yunge L (1979). Ultrastructural cytochemistry of atrial muscle cells. V. Comparative study of specific granules in right and left atrium of various animal species. Anat Rec 193: 55-70.
Cantin M, Gutkowska J, Thibault G, Milne RW, Ledoux S, Minli S, Chapeau C, Garcia R, Hamet P, Genest J (1984). Immuno-cytochemical localization of atrial natriuretic factor and salivary glands. Histochem 80: 113-27.
Cantin M (1985). Localization of immunoreactive synthetic atrial natriuretic factor (ANF) in the heart of various animal species. J Histochem Cytochem 33(6): 541-550.
Cantin M, Genest J (1985). The heart and the atrial natriuretic factor. Endocr Rev 6:107-127.
Cea LB (2005). Natriuretic petide family: new aspects. Curr Med Chem Cardiovasc Hematol Agents 3:87-98.
Chapeau C, Gutkowska J, Schiller PW, Milne RW, Thibault G, Garcia R, Genest J, Cantin M (1985). Localization of immunoreactive synthetic atrial natriuretic factor (ANF) in the heart of various animal species. J Histochem Cytochem 33(6): 541-550.
Chiu IS, Chiang FT, How SW (1994). Atrial natriuretic peptide is produced less in the intercaval sinus than in the pectinated right atrium. J Thorac Cardiovasc Surg 42: 158-161.
Cho KW, Seul KH, Kin SH, Seul KM, Koh GY (1991). Atrial pressure, distention, and pacing frequency in ANP secretion in isolated perfused rabbit atria. Am J Physiol, 260: R39-46.
Cox JL, Boineau JP, Schuessler RB (1991). Successful surgical treatment of atrial fibrilation. JAMA, 266: 1976-80.
De Bold AJ, Borenstein HB, Veress AT, Sonnenberg H (1981). A rapid and potent natriuretic response to intravenous injection of atrial myocardial extracts in rat. Life Sci 28: 89-94.
De Bold AJ, Ma KK, Zhang Y, de Bold ML, Bensimon M, Khoshbaten A (2001). The physiological and pathophysiological modulation of the endocrine function of the heart. Can. J. Physiol. Pharmacol. 79:705-714.
De Bold AJ, de Bold ML (2005). Determinants of natriuretic peptide production by heart: basic and clinical implications. J Investig Med 53: 371-377.
Dietz JR (2005). Mechanisms of atrial natriuretic peptide secretion from the atrium Cardiovasc Res 68: 8-17.
Forssmann WG, Birr C, Carlquist M, Christmann M, Finke R, Henschen A, Hock D, Kischheim H, Kreye V, Lottspeich F, Metz J, Mutt J, Reincke M (1984). The auricular myocardiocytes of the heart constitute an endocrine organ. Cell Tissue Res 238: 425-430.
Gall JAM, Alcorn D, Ferney R, Coghlan JP, Ryan GB (1990). Qualitative and quantitative analysis of granules in atrial appendage cardiocytes in different physiological states. Cell Tissue Res 259: 529-534.
Gama EF, Ferraz de Carvalho CA, Liberti EA, De Souza RR Natriuretic peptide (NP)-granules in the guinea pig atrial and auricular cardiocytes: an immunocytochemical and ultrastructural morphometric comparative study. Ann Anat (in press)
Gilloteaux J, Jennes L, Menu R, Vanderhaeghen JJ (1991). Ultrastructural immunolocalization of the atrial natriuretic factor pathways in fetal, neonatal, and adult Syrian hamsters: from the atrial cardiomyocytes to the circulation via the endocardium, atrial capillaries and epicardium. J Submicrosc Cytol Pathol 23 (1): 75-91.
Horio T, Kohno M, Takeda T (1993) Cosecretion of atrial and brain natriuretic peptides stimulated by endothelin-1 from cultured rat atrial and ventricular cardiocytes. Metabolism 42: 94-6.
Imada T, Takayamagi K, Inagami T (1988). Atrioactivase, a specific peptidase in bovine atria for the processing of proatrial natriuretic factor. Purification and characterization. J Biol Chem 263: 9515-19.
Jamieson JD, Palade GE (1964). Specific granules in atrial muscle cells. J Cell Biol 123:151-172.
Jiao JH, Guyenet PG, Baertschi AL (1993). Lower brain stem controls cardiac ANF secretion. Am J Physiol 263: H198-207.
Katoh S, Toyama J, Aoyama M, Miyamoto N, Sco H, Matsui N, Kodama J, Yamada K (1990). Mechanisms of atrial natriuretic peptide (ANP) secretion by rat hearts perfused *in vitro*-Ca_2(+)-dependent signal transduction for ANP release by mechanical stretch. Jpn Circ J 54: 1283-1294.
Kish B (1956). Electron Microscopy of the Atrium of the Heart. I. Guinea Pig. Exp Med Surg 14: 99-112.
Klinger JR, Warburton R, Carino GP, Murray J, Murphy C, Napier M, Harrington EO (2005). Natriuretic peptides differentially attenuate thrombin-induced barrier dysfunction in pulmonary microvascular endothelial cells. Exp Cell Res [Epub ahead of print].
McCarthy PM, Castle LM, Maloney JD (1993) Initial experience with the maze procedure for atrial fibrilation. J Thorac Cardiovasc Surg 105: 1077-87.
Michels P, Tarnow J (2001). Natriuretic peptides: physiological , pathophysiolocal and clinical aspects. Anasthesiol Intensivmed Notfallmed Schmertzther 36: 406-416.
Mifune H, Suzuki S, Noda Y, Mohri S, Mochizuki K (1991). Fine structure of atrial natriuretic peptide (ANP)- granules in the atrial cardiocytes in the pig, cattle and horse. J Vet Med Sci 53: 561-568.
Mifune H, Suzuki S, Noda Y, Hayashi Y, Mochizuki K (1992). Fine structure of atrial natriuretic peptide (ANP)-granules in the atrial cardiocytes in the hamster, guinea pig, rabbit, cat and dog. Jikken Dobutsu 41: 321-8.
Mifune H, Suzuki S, Nokihara K, Kobayashi Y, Ueda T, Obara T, Noda Y, Sakamoto H (1993). Effects of environmental temperature on atrial natriuretic peptide (ANP)–granules of auricular cardiocytes and plasma ANP level in pregnant rats. Jikken Dobutsu 42: 243-247.
Mifune H, Suzuki S, Nokihara K, Noda Y (1996). Distribution of immunoreactive atrial and brain natriuretic peptides in the heart of the chicken, quail, snake and frog. Exp Anim 45:125-133.
Mifune H, Honda J, Takamori S, Sugiyama F, Yagami K, Suzuki S (2004). A-type natriuretic peptide level in hypertensive transgenic mice. Exp Anim 53: 11-19.
Nakayama K, Ohkubo H, Hirose T, Inayama S Nakanishi S (1984). mRNA sequence for human cardiodilatin-atrial natriuretic factor precursor and regulation of precursor mRNA in rat atria. Nature 23: 699-701.
Needleman P, Blaine EH, Greenwald JE, Michener ML, Saper CB, Stockmann PT, Tolunay HE (1989) The biochemical pharmacology of atrial peptides. Ann Ver Pharm Toxicol 29: 25-54.
Penner SB, Thliveris JA, McKenzie JK, Smyth DD (1990). Atrial-specific granule number and plasma atrial natriuretic peptide in rats: effects of beta-adrenoceptor blockade and sodium intake. Anat Rec 228: 418-424.
Rinne A, Vuolteenaho O, Järvinen, Dorn A, Arjamaa O (1986). Atrial natriuretic polypeptides in the specific atrial granules of the rat heart: immunohistochemical and immunoelectron microscopical localization and radioimmunological quantification. Acta Histochem 80: 19-28.
Schiebinger RJ, Greening KM (1992). Interaction between stretch and hormonally stimulated atrial natriuretic peptide secretion. Am J Physiol 262: H78-H83.
Seul KH, Cho KW, Kim SH (1992). Right atrial predominance of atrial natriuretic peptide secretion in isolated perfused rat atria. Regulatory Peptides 39: 67-81.
Silver MA (2006). The natriuretic peptide system: kidney and cardiovascular effects. Curr Opin Nephol Hypertens 15: 14-21.
Skepper JN, Navaratman V (1988). Analysis of the apparent heterogeneity of specific heart granules in rat atrial myocytes; an ultrastructural study including immunocytochemistry. Histochem J 20: 1-10.
Skepper JN, Navaratman V, Martensz ND (1989). Effects of expansion of blood volume and bilateral vagotomy on specific heart granules and release of atrial natriuretic peptide in the rat. Cell Tissue Res 258: 211-218.
Stewart JM, Dean R, Brown M, Diasparra D, Zeballos GA, Schustek, M, Gewitz MH, Thompson CL, Hintze TH (1992). Bilateral atrial appendectomy abolishes increased plasma atrial natriuretic peptide release and blunts sodium and water excretion during volume loading in conscious dogs. Circ Res 70: 724-32.
Takayanagi R, Tanaka I, Maki M, Inagami T (1985). Effects of changes in water sodium balance on levels of atrial natriuretic factor messenger RNA and peptide in rats. Life Sci 36: 1843-1848

Tanaka H, Shindo M, Gutkowska J, Kinoshita A, Urata H, Ikeda M, Arakawa K (1986). Effect of acute exercise on plasma immunoreactive atrial natriuretic factor. Life Sci 39: 1685-1693.

Toyoshima Y, Suzuki S, Awal MA, Matsumoto M, Nishinakagawa H, Mifune H, Honda J (1996). Atrial natriuretic peptide (ANP)-granules of auricular cardiocytes in dehydrated and rehydrated mice. Exp Anim 45: 135-140.

Veress AT, Sonnerberg H (1984). Right atrial appendectomy reduces the renal response to acute hypervolemia in the rat. Am J Physiol 247: R610-R613.

Yoshihara F, Nashikimi T, Kosakai Y, Isobe F, Matsuoka H, Takishita S, Kawashima Y, Saito Y, Matsuo H, Kangawa K (1998). Atrial natriuretic peptide secretion and body fluid balance after bilateral atrial appendectomy by the maze procedure. J Thorac Cardiovasc Surg 116(2): 213-9.