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β-Adrenoceptor antagonist propranolol potentiates hypotensive action of natriuretic peptides

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Abstract

β-Adrenoceptor antagonists are known to increase plasma atrial natriuretic peptide (ANP) levels despite their hypotensive action. The aim of the present study was to examine the role of the ANP system in the antihypertensive effects of a β-adrenoceptor antagonist. We investigated the effects of propranolol (75 mg kg^{-1} day^{-1}, p.o., 4 weeks) on the ANP system in stroke-prone spontaneously hypertensive rats. Plasma ANP levels were significantly higher in the propranolol group than in the control group. Both receptor densities and mRNA levels of ANP_C receptor were significantly decreased in the lung as the major site of ANP clearance from the circulation. In contrast, both central venous pressure and ANP mRNA levels in the heart were not significantly different between the two groups. Under both basal and ANP-stimulated conditions, the cGMP content in the aorta was significantly greater in the propranolol group than in the control group, whereas the basal and stimulated cGMP content of the kidney was similar in the two groups. Inhibition of endogenous ANP action by a specific ANP receptor antagonist, HS-142-1, produced a greater increase of blood pressure in the propranolol group than in the control group. These results suggest potentiation of natriuretic peptide activity as a new antihypertensive mechanism of the β-adrenoceptor antagonist propranolol.

Keywords: Natriuretic peptide; β-Adrenoceptor antagonist; Hypertension; ANP receptor

1. Introduction

β-Adrenoceptor antagonists are now classified as one of the first-line medicines for the treatment of hypertension (Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure, 1988). Although various mechanisms have been suggested to be responsible for the antihypertensive action, no conclusive view has been reached (for review: Cruickshank and Prichard, 1994). Natriuretic peptide (NP) has been demonstrated to play an important role in cardiovascular homeostasis (for review: Rosenzweig and Seidman, 1991; Maack, 1992). Plasma atrial natriuretic peptide (ANP) levels are increased in patients with hypertension and decrease to normal levels after treatment with various antihypertensive agents, mainly through a reduction of the cardiac afterload (Knorr et al., 1991; Phillips et al., 1991; Cappuccio et al., 1991; Nishimura et al., 1992). Intriguing is the finding that treatment with β-adrenoceptor antagonists increases plasma ANP levels despite causing a significant decrease in blood pressure (Nakaoka et al., 1987; Yamamoto et al., 1989; Tonkin et al., 1990; Hollenbeck et al., 1991). The mechanism and the pathophysiological significance of the phenomenon, however, remain to be elucidated.

The aim of the present study was to examine the role of the ANP system in the antihypertensive effects of a β-adrenoceptor antagonist. We investigated the effects of propranolol as one of the most representative β-adrenoceptor antagonists on plasma ANP levels, cardiac ANP mRNA expression, ANP receptor expression and function, and antihypertensive action of endogenous ANP in stroke-prone spontaneously hypertensive rats (SHR-SP/Izm).

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2. Materials and methods

2.1. Animals and treatments

Male SHR-SP/Izm rats (Disease Model Cooperative Research Association, Kyoto, Japan) (Nabika et al., 1991) of 12 weeks of age were divided into two groups: the group treated with propranolol (75 mg kg^{-1} day^{-1}, p.o.) suspended in 0.5% methylcellulose solution and the control group treated with 0.5% methylcellulose solution alone as vehicle for 4 weeks. All following experiments were performed after this 4-week treatment. Systolic blood pressure and heart rate were recorded on the day before death by the tail-cuff method as described previously (Yoshimoto et al., 1995).

2.2. Measurements of plasma ANP

Plasma ANP concentrations were determined by using commercially available radioimmunoassay (RIA) kits specific for α-rat ANP (Peninsula, Belmont, CA) as described previously (Yoshimoto et al., 1996a).

2.3. Measurements of central venous pressure (CVP)

The right atrium was cannulated with polyethylene catheters (PE-50) through the right jugular vein in both groups of rats anesthetized with 20% urethane (5 ml kg^{-1} i.p.). Mean central venous pressure was measured by a transducer (Model MPU-0.5A, Nihon Kohden Kogyo, Tokyo, Japan) with an amplifier (Model AP-621G, Nihon Kohden Kogyo).

2.4. Ribonuclease (RNAase) protection assay for ANP and its receptor mRNAs

Total RNA extracts from atrium, left ventricle, aorta, kidney and lung were obtained by the acid guanidinium–thiocyanate–phenol–chloroform method (Chomczynski and Sacchi, 1987). Right and left atria were combined for RNA extraction from the atria. Left ventricle with interventricular septum was used for RNA extraction from the ventricle. mRNA levels of the ANPA receptor as a biologically active subtype were determined in the aorta and kidney and mRNA levels of the ANP_C receptor as a clearance receptor were determined in the aorta, kidney, and lung by RNAase protection assay as described previously (Yoshimoto et al., 1995, 1996b). ANP mRNA levels in the cardiac tissue were determined also in an RNAase protection assay (Yoshimoto et al., 1995, 1996b), using antisense cRNA probes for rat ANP corresponding to 244–475 bp of rat ANP cDNA sequence (Kangawa et al., 1984). Briefly, a 20-μg sample of RNA was hybridized with ^{32}P-labeled antisense probe (1×10^5 cpm) overnight at 55°C, followed by digestion with ribonuclease, electrophoresis, and exposure to X-ray films. The optical density of the radioactive bands was determined densitometrically and was normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in each sample, as described previously (Yoshimoto et al., 1995, 1996b).

2.5. ANP binding assay for determination of ANPc receptor density in lung

The specific activity of [^{125}I]α-rat ANP (74 TBq $mmol^{-1}$; Amersham, Buckinghamshire, UK) was adjusted with unlabeled α-rat ANP (Peptide Institute, Osaka, Japan) to 1000–1100 mCi μmol^{-1}. The crude membrane fraction from lung (30 μg of protein) was incubated in duplicate for 90 min at 25°C with a saturable concentration (0.14 nM) of [^{125}I]α-rat ANP (1000–1100 mCi μmol^{-1}) in the presence or absence of an excess amount of unlabeled des[Gln 18, Ser 19, Gly 20, Leu 21, Gly 22] ANP-(4-23)-NH_2 (C-ANF) (1 μM) (Peninsula). C-ANF is a synthetic ring-deleted analog of ANP that binds specifically to the ANP_C receptor (Maack et al., 1987). To determine the specific binding to the ANP_C receptor, [^{125}I]α-rANP binding in the presence of excess C-ANF was subtracted from the total binding as described by Kishimoto et al. (1994).

2.6. Cyclic nucleotide content in the aorta and the kidney in response to ANP

The aortic strip (approximately 20–30 mg) and kidney slices of 1-mm thickness were suspended in Krebs–Ringer phosphate buffer, pH 7.4, containing 0.2 mM isobutylmethylxanthine (Sigma) at 37°C under bubbling with a mixture of 95% O_2 and 5% CO_2. Then, tissues were exposed to 10^{-8} M α-rat ANP for 10 min. The reaction was stopped by immersion of the tissues in liquid nitrogen. Frozen tissues were homogenized with 2 ml of ice-cold 6% perchloric acid, which was removed by neutralization with 0.2 ml of 60% KOH. cGMP contents were measured by using a commercially available RIA kit after a succinylation step (Yamasa Shoyu, Choshi, Japan).

2.7. Effects of ANP receptor antagonist, HS-142-1, on systolic blood pressure

The biological significance of endogenous ANP in the maintenance of blood pressure was investigated by intraperitoneal bolus injection of specific ANPA/ANPB receptor antagonist, HS-142-1 (a polysaccharide composed of 20–30 kinds of linear b-1, 6 glucan esterified by caproyl groups) (8 mg kg body $weight^{-1}$) (for review, see Matsuda, 1997), as described previously (Yoshimoto et al., 1996a). Systolic blood pressure was recorded by the tail-cuff method before and 30 min and 60 min after administration of HS-142-1. For the tail-cuff measurements, at least six measurements were made at each time point in each rat and the average values were used for statistical analyses.

Table 1
Systolic blood pressure, heart rate and plasma ANP levels in the control and propranolol groups of SHR-SP/Izm

	Control	Propranolol
Systolic blood pressure (mmHg)	226 ± 3 (5)	194 ± 3 (5)[a]
Heart rate (beats/min)	394 ± 5 (5)	340 ± 7 (5)[a]
Plasma ANP levels (pg/ml)	168 ± 5 (5)	199 ± 6 (5)[a]

Values were expressed as the mean ± S.E.M. Numbers of experiments were shown in parentheses.
[a] $P < 0.05$ vs. control group.

2.8. Statistical analysis

Values are expressed as the means ± S.E.M. of five different experiments, except values of mRNA analysis, which are expressed as the means ± S.E.M. of four different experiments. Differences between the two groups were analyzed by the Mann–Whitney *U*-test. Changes in the systolic blood pressure in the studies with HS-142-1 were analyzed by one-way analysis of variance followed by Dunnett's test. *P*-values less than 0.05 were considered statistically significant.

3. Results

Both systolic blood pressure and heart rate were significantly decreased and plasma ANP levels were significantly increased in the propranolol group (Table 1). However, there was no significant change in the ANP mRNA levels of the atrium and left ventricle in the propranolol group compared with the control group (Fig. 1a). There was no significant difference in the mean CVP between the two groups (the vehicle group: 4.8 ± 0.7 mmHg, $n = 5$; the propranolol group: 5.0 ± 0.9 mmHg, $n = 5$).

The representative autoradiograms of the RNAase protection assay for both ANPA and ANP_C receptors are shown in Fig. 1b and c. There was no significant difference in the levels of ANPA receptor mRNA in the aorta and kidney between the control and propranolol groups. However, the ANP_C receptor mRNA levels were significantly downregulated in the aorta (control: 6.6 ± 0.4 attomol 20 μg RNA^{-1}, $n = 4$; propranolol: 4.1 ± 0.5 attomol 20 μg RNA^{-1}, $n = 4$, $P < 0.05$) and the lung (control: 110 ± 10 attomol 20 μg RNA^{-1}, $n = 4$; propranolol: 67.8 ± 4.7 attomol 20 μg RNA^{-1}, $n = 4$, $P < 0.05$) of the propranolol group compared with the control group, while there was no significant difference in the kidney between the two groups. In addition, the binding capacity of the ANP_C receptor in the lung was significantly ($P < 0.05$) decreased in the propranolol group (38 ± 3.7 fmol mg $protein^{-1}$, $n = 5$) compared with the control group (75 ± 10 fmol mg $protein^{-1}$, $n = 5$).

Under basal (0.72 ± 0.08 pmol mg $protein^{-1}$, $n = 5$) and ANP-stimulated (8.6 ± 0.7 pmol mg $protein^{-1}$, $n = 5$)

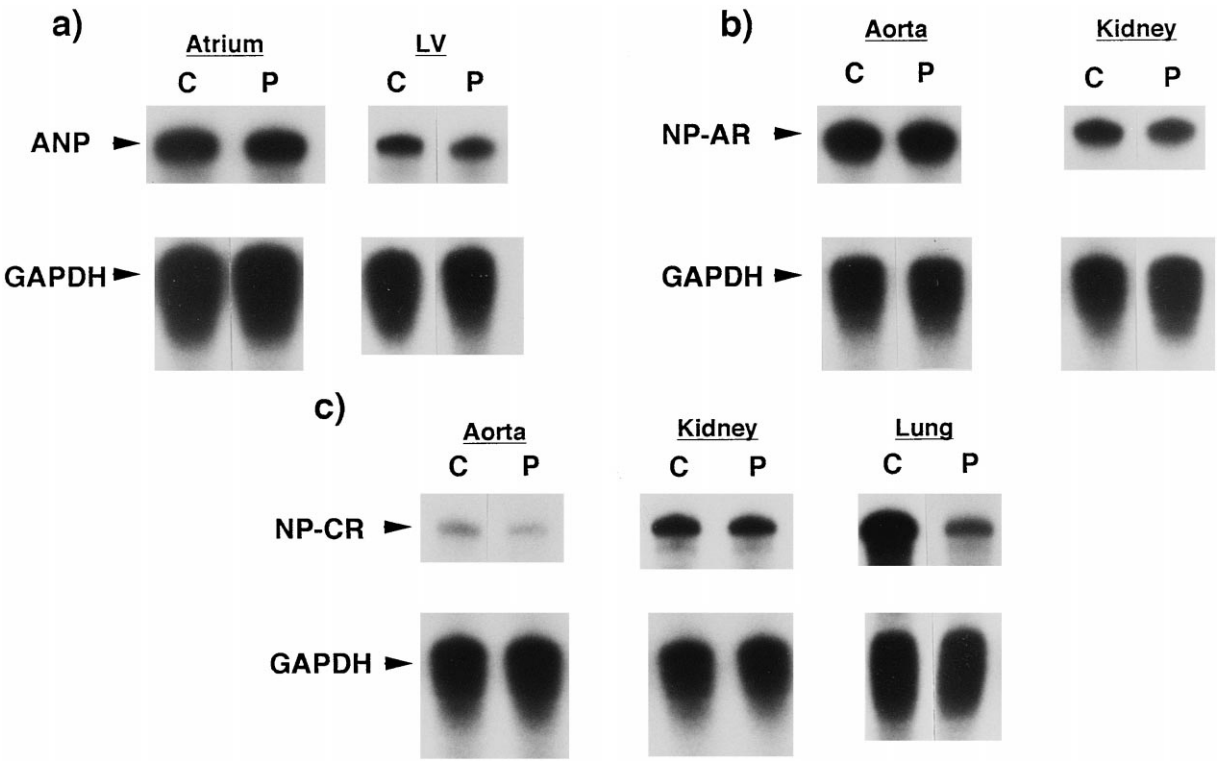

Fig. 1. Typical autoradiogram of polyacrylamide gel electrophoresis of mRNA for ANP (a), ANPA receptor (b), and ANP_C receptor (c), as determined in the RNAase protection assay. Atrial RNA was extracted from the combined tissue of right and left atria. Ventricular RNA was extracted from the tissue of the left ventricle with interventricular septum. C, control group; P, propranolol group; LV, left ventricle; NP-AR, ANPA receptor; NP-CR, ANP_C receptor.

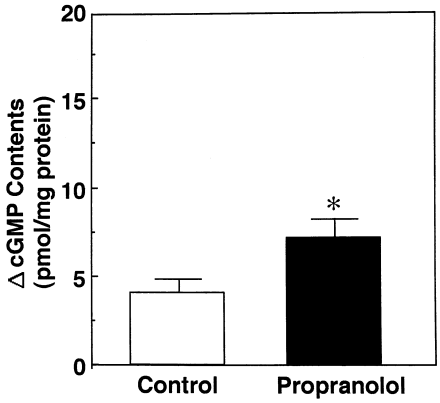

Fig. 2. Changes in the cGMP content of the aorta in response to ANP (10^{-8} M) in the control and propranolol groups of SHR-SP/Izm. Values are the means ± S.E.M. of five different experiments. * $P < 0.05$ vs. control group.

conditions, the cGMP content in the aortic strip was significantly ($P < 0.05$) increased in the propranolol group compared with the control group (basal: 0.39 ± 0.04 pmol mg protein^{-1}; ANP-stimulated: 4.6 ± 0.8 pmol mg protein^{-1}, $n = 5$). The change in the cGMP content of the aorta after addition of ANP is shown in Fig. 2. Under basal (26 ± 4 fmol mg protein^{-1}, $n = 5$) and ANP-stimulated (126 ± 19 fmol mg protein^{-1}, $n = 5$) conditions, the cGMP contents in the kidney slice of the propranolol group was comparable to that of the vehicle group (basal: 23 ± 6 fmol mg protein^{-1}; ANP-stimulated: 111 ± 23 fmol mg protein^{-1}, $n = 5$).

Administration of HS-142-1, a specific antagonist of ANPA/ANPB receptors, produced a transient but significant ($P < 0.05$) increase of systolic blood pressure in both control and propranolol groups (Fig. 3). The magnitude of the increase in blood pressure was significantly ($P < 0.05$) greater in the propranolol group (16 ± 3 mmHg, $n = 5$) than in the control group (8 ± 3 mmHg, $n = 5$).

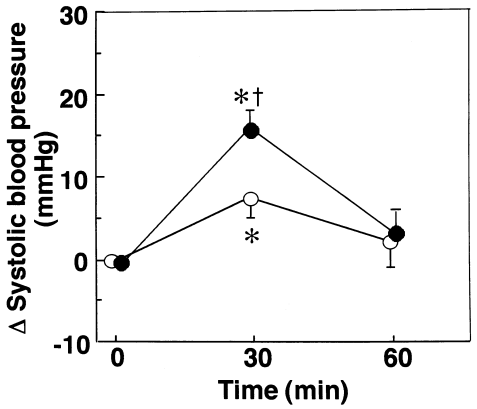

Fig. 3. Effects of the ANP-A/ANP-B receptor antagonist, HS-142-1, on systolic blood pressure in the control (open circle) and propranolol (closed circle) groups of SHR-SP/Izm. Changes in the systolic blood pressure from the baseline values are shown. Values are the means ± S.E.M. of five different experiments. * $P < 0.05$ vs. baseline value; † $P < 0.05$ vs. control group.

4. Discussion

In agreement with previous reports (Nakaoka et al., 1987; Yamamoto et al., 1989; Tonkin et al., 1990; Hollenbeck et al., 1991), plasma ANP levels were significantly elevated in the propranolol group. Plasma ANP levels are primarily determined by ANP synthesis and secretion from the heart (Rosenzweig and Seidman, 1991). Chronic treatment with propranolol may increase cardiac filling pressure due to its negative innotropic effect, leading to an enhanced ANP secretion. However, mean CVP and cardiac ANP mRNA levels, which have been shown to correlate with cardiac ANP synthesis (Arai et al., 1988; Saito et al., 1989), were not significantly different in the propranolol group and in the vehicle group. It is therefore unlikely that chronic treatment with propranolol increases plasma ANP levels by increasing cardiac filling pressure and then ANP secretion.

Another important factor that affects plasma ANP levels is the clearance of ANP from the circulation through its receptors. There are three subtypes of ANP receptor: ANPA, ANPB and ANP_C receptors. Although both ANPA and ANPB receptors have an intrinsic guanylate cyclase domain and exert biological actions through cGMP formation (Maack, 1992), the ANPA receptor is the specific and predominant subtype for ANP and BNP as circulating hormones (Maack, 1992). In contrast, the ANP_C receptor has been considered to be the clearance receptor for NPs (Maack et al., 1987; Nussenzveig et al., 1990). We therefore determined the mRNA levels of ANPA and ANP_C in tissues. There was no significant difference in the levels of ANPA receptor mRNA between the two groups. However, the ANP_C receptor mRNA levels were significantly down-regulated in the aorta and the lung of the propranolol group compared with the control group. In addition, the binding capacity of the ANP_C receptor in the lung was significantly decreased in the propranolol group compared with the control group. Since the pulmonary circulation receives nearly the entire cardiac output and the lung is the major site of ANP clearance from the circulation (Klinger et al., 1992; Hollister et al., 1989; Perrella et al., 1991), the decreased ANP_C receptor mRNA expression and receptor density in the lung of the propranolol group may cause a decreased clearance of ANP from the circulation, leading to an elevation of plasma ANP levels.

Since the regulation of ANP_C receptor expression in renal tissue is not fully understood, it remains to be seen whether there is some other mechanism regulating ANP_C receptor expression in the kidney which is different from that in the vessels and lung. Renal tissues consist of heterogeneous nephron segments and interstitial tissues

and we used RNA extracted from the whole kidney tissue in the present study. Changes in ANP_C receptor mRNA expression in a certain portion could be masked by changes in other portions. Details of the effect of propranolol on ANP_C receptors in each nephron segment of the kidney remain to be determined.

Whether the downregulated ANP_C receptor and increased plasma ANP concentration are related to an enhancement of the biological activity of the ANP system was ascertained in ex vivo and in vivo experiments. Under both basal and ANP-stimulated conditions, the cGMP content in the aortic strip was significantly increased in the propranolol group compared with the control group. Ang II, which downregulates the ANP_C receptor (Yoshimoto et al., 1996b), was previously reported to exaggerate the cGMP response to ANP in rat cultured vascular smooth muscle cells (Chabrier et al., 1988). Locally decreased clearance of ANP through the ANP_C receptor may increase the availability of ANP for the ANPA receptor, leading to an enhancement of the cGMP response to ANP. In addition, administration of HS-142-1, a specific antagonist of the ANPA/ANPB receptor, produced a greater increase of systolic blood pressure in the propranolol group than in the control group. This result suggests that the hypotensive action of endogenous ANP is potentiated by propranolol treatment.

All these findings suggest that the β-adrenoceptor antagonist propranolol potentiates the action of ANP by increasing plasma ANP levels and by enhancing the vascular response to ANP through the downregulation of the ANP_C receptor. The novel mechanism seems to account for a sizable portion of the antihypertensive effect of propranolol and could be of potential importance in the treatment of cardiovascular disease with β-adrenoceptor antagonists.

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References

Arai, H., Nakao, K., Saito, Y., Morii, N., Sugawara, A., Yamada, T., Itoh, H., Shiono, S., Mukoyama, S., Ohkubo, H., Nakanishi, S., Imura, H., 1988. Augmented expression of atrial natriuretic polypeptide in ventricles of spontaneously hypertensive rats (SHR) and SHR stroke-prone. Circ. Res. 62, 926–930.

Cappuccio, F.P., Markandu, N.D., Sagnella, G.A., Singer, D.R., Buckley, M.G., 1991. Effects of amlodipine on urinary sodium excretion, renin–angiotensin–aldosterone system, atrial natriuretic peptide, and blood pressure in essential hypertension. J. Hum. Hypertens. 5, 115–119.

Chabrier, P.E., Roubert, P., Lonchampt, M.O., Plas, P., Braquet, P., 1988. Regulation of atrial natriuretic factor receptors by angiotensin II in rat vascular smooth muscle cells. J. Biol. Chem. 263, 13199–13202.

Chomczynski, P., Sacchi, N., 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. Anal. Biochem. 162, 156–159.

Cruickshank, J.M., Prichard, B.N.C., 1994. Beta-blockers. In: Swales, J.D. (Ed.), Textbook of Hypertension. Blackwell, Oxford, pp. 1067–1069.

Hollenbeck, M., Plum, J., Heering, P., Kutkuhn, B., Grabensee, B., 1991. Influence of betaxolol on renal function and atrial natriuretic peptide in essential hypertension. J. Hypertens. 9, 819–824.

Hollister, A.S., Rodeheffer, R.J., White, F.J., Potts, J.R., Imada, T., Inagami, T., 1989. Clearance of atrial natriuretic factor by lung, liver, and kidney in human subjects and the dog. J. Clin. Invest. 83, 623–628.

Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure, 1988. The 1988 report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure. Arch. Intern. Med. 148, 1023–1038.

Kangawa, K., Tawaragi, Y., Oikawa, S., Mizuno, A., Sakuragawa, Y., Nakazato, H., Fukuda, A., Minamino, N., Matsuo, H., 1984. Identification of rat gamma atrial natriuretic polypeptide and characterization of the cDNA encoding its precursor. Nature 312, 152–155.

Kishimoto, I., Yoshimasa, T., Suga, S., Ogawa, Y., Komatsu, Y., Nakagawa, O., Itoh, H., Nakao, K., 1994. Natriuretic peptide clearance receptor is transcriptionally down-regulated by β2-adrenergic stimulation in vascular smooth muscle cells. J. Biol. Chem. 269, 28300–28308.

Klinger, J.R., Moalli, R., Warburton, R.R., Wrenn, D.S., Hill, N.S., 1992. C-receptor ligand blocks pulmonary clearance of atrial natriuretic peptide in isolated rat lung. Proc. Soc. Exp. Biol. Med. 201, 154–158.

Knorr, A., Kazda, S., Neuser, D., Stasch, J.P., Seuter, F., 1991. Long-term protective effects of nitrendipine in experimental hypertension. J. Cardiovasc. Pharmacol. 18 (Suppl. 5), S1–S4.

Maack, T., 1992. Receptors of atrial natriuretic factor. Annu. Rev. Physiol. 54, 11–27.

Maack, T., Suzuki, M., Almedia, F.A., Nussenzveig, D., Scarborough, R.M., McEnroe, G.A., Lewicki, J.A., 1987. Physiological role of silent receptors of atrial natriuretic factor. Science 238, 675–678.

Matsuda, Y., 1997. Design and utilization of natriuretic peptide antagonists. In: Samson, W.K., Levin, E.R. (Eds.), Contemporary Endocrinology: Natriuretic Peptides in Health and Disease. Humana Press, Totowa, NJ, pp. 289–307.

Nabika, T., Nara, Y., Ikeda, K., Endo, J., Yamori, Y., 1991. Genetic heterogeneity of the spontaneously hypertensive rat. Hypertension 18, 12–16.

Nakaoka, H., Kitahara, Y., Amano, M., Imataka, K., Fujii, J., Ishibashi, M., Yamaji, T., 1987. Effects of β-adrenergic receptor blockade on atrial natriuretic peptide in essential hypertension. Hypertension 10, 221–225.

Nishimura, H., Kubota, J., Okabe, M., Ueyama, M., Kawamura, K., 1992. Effects of lisinopril upon cardiac hypertrophy, central and peripheral hemodynamics, and neurohumoral factors in spontaneously hypertensive rats. J. Hypertens. 10, 431–436.

Nussenzveig, D.R., Lewicki, J.A., Maack, T., 1990. Cellular mechanisms of the clearance function of type C receptors of atrial natriuretic factor. J. Biol. Chem. 265, 20952–20958.

Perrella, M.A., Margulies, K.B., Wei, C.M., Aarhus, L.L., Heublein, D.M., Burnett Jr., J.C., 1991. Pulmonary and urinary clearance of atrial natriuretic factor in acute congestive heart failure in dogs. J. Clin. Invest. 87, 1649–1655.

Phillips, R.A., Ardeljan, M., Shimabukuro, S., Goldman, M.E., Garbowit, D.L., Eison, H.B., Krakoff, L.R., 1991. Normalization or left ventricular mass and associated changes in nifedipine therapy for severe hypertension. J. Am. Coll. Cardiol. 17, 1595–1602.

Rosenzweig, A., Seidman, C.E., 1991. Atrial natriuretic factor and related peptide hormones. Annu. Rev. Biochem. 60, 229–255.

Saito, Y., Nakao, K., Arai, H., Nishimura, K., Okumura, K., Obata, K., Takemura, G., Fujiwara, H., Sugawara, A., Yamada, T., Itoh, H., Mukoyama, M., Hosoda, K., Kawai, C., Ban, T., Yasue, H., Imura, H., 1989. Augmented expression of atrial natriuretic polypeptide gene in ventricle of human failing heart. J. Clin. Invest. 83, 298–305.

Tonkin, A.L., Wing, L.M.H., Russell, A.E., West, M.J., Bune, A.J.C., Morris, M.J., Cain, M.D., Chalmers, J., 1990. Diltiazem and atenolol in essential hypertension: additivity of effects on blood pressure and cardiac conduction with combination therapy. J. Hypertens. 8, 1015–1019.

Yamamoto, J., Matsubara, H., Nakai, M., 1989. Effects of arotinolol on hemodynamics and plasma atrial natriuretic peptide at rest and during stress in spontaneously hypertensive rats. Clin. Exp. Hypertens. A 11, 1441–1454.

Yoshimoto, T., Naruse, M., Naruse, K., Fujimaki, Y., Tanabe, A., Muraki, T., Itakura, M., Hagiwara, H., Hirose, S., Demura, H., 1995. Modulation of vascular natriuretic peptide receptor gene expression in hypertensive and obese hyperglycemic rats. Endocrinology 136, 2427–2434.

Yoshimoto, T., Naruse, M., Naruse, K., Arai, K., Imaki, T., Tanabe, A., Seki, T., Hirose, S., Muraki, T., Demura, H., 1996a. Vascular action of circulating and local natriuretic peptide systems is potentiated in obese/hyperglycemic and hypertensive rats. Endocrinology 137, 5552–5557.

Yoshimoto, T., Naruse, M., Naruse, K., Shionoya, K., Tanaka, M., Tanabe, A., Hagiwara, H., Hirose, S., Muraki, T., Demura, H., 1996b. Angiotensin II-dependent down-regulation of vascular natriuretic peptide receptor gene expression in hypertensive rats. Endocrinology 137, 1102–1107.