Effects of Homologous Atrial, Brain, and C-type Natriuretic Peptides on Isolated Heart and Blood Vessels of Bullfrog

Authors: Uchiyama, Minoru, Murayama, Tomona, Matsuda, Kouhei, Watanabe, Takushs X., and Takei, Yoshio

Source: Zoological Science, 14(5) : 843-847

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.14.843

ZOOLOGICAL SCIENCE 14: 843–847 (1997)

Effects of Homologous Atrial, Brain, and C-type Natriuretic Peptides on Isolated Heart and Blood Vessels of Bullfrog

Minoru Uchiyama[1]*, Tomona Murayama[1], Kouhei Matsuda[1], Takushi X. Watanabe[2] and Yoshio Takei[3]

[1]*Department of Biology, Faculty of Science, Toyama University, 3190 Gofuku, Toyama 930, Japan*
[2]*Peptide Institute Inc., Protein Research Foundation, Minoh, Osaka 562, Japan*
[3]*Ocean Research Institute, University of Tokyo, Nakano, Tokyo 164, Japan*

ABSTRACT—The cardiovascular effects of homologous natriuretic peptides (fNPs) were examined in the bullfrog, *Rana catesbeiana*. Synthetic bullfrog atrial natriuretic (fANP), brain natriuretic (fBNP) and C-type natriuretic peptides (fCNP I and fCNP II), were tested *in vitro* and compared under the same experimental conditions. All frog NPs produced a significant, and concentration-dependent, reduction in tension (relaxant effect) in the isolated dorsal aorta. Frog CNP II exhibited similar vasorelaxation profile as that of fANP, while fBNP and fCNP I had lower activity than fANP. Frog NPs inhibited norepinephrine induced contraction and fCNP II was most potent. In isolated preparations of atrium and ventricle, fCNP I and II produced a significant, and concentration-dependent, reduction in tension, but neither fANP nor fBNP produced any significant effects. Frog CNP II is most potent among fNPs in relation to reduce the cardiac output. Chronotropic responses of the heart to administrations of NPs were insignificant. The present results for the first time showed that fNPs play roles in the control of cardiovascular system, both blood vessels and heart, in the bullfrog.

INTRODUCTION

In mammals, the natriuretic peptides (NPs) family, atrial natriuretic (ANP), brain natriuretic (BNP) and C-type natriuretic peptide (CNP) groups, has a high sequence homology, particularly within the 17-amino acid ring formed between two cysteine residues. While NPs exhibit biological functions through a direct activation of guanosine 3', 5'-cyclic monophosphate (cGMP), ANP and BNP activate natriuretic peptide receptor (NPR)-A, whereas CNP binds with NPR-B at a high affinity level (Koller *et al.*, 1991; Hagiwara *et al.*, 1995). Recently NPs have been purified and sequenced from brain (CNP I) and heart (ANP and BNP) or were cloned (CNP II) from brain cDNA library in the bullfrog (Sakata *et al.*, 1988; Yoshihara *et al.*, 1990; Kojima *et al.*, 1994; Fukuzawa *et al.*, 1996).

Since the discovery of ANP by de Bold *et al.* (1981), attention has been focused on the control of the cardiovascular system in vertebrates, where NPs produce dilation of vascular smooth muscle, both *in vivo* and *in vitro* (see Brenner *et al.*, 1990; Winquist and Hintze, 1990). However, CNP has been shown to have very little hypotensive effect in rats when compared to ANP or BNP (Sudoh *et al.*, 1990). Indeed, CNP primarily functions as a neuromodulator and a growth inhibitor of cell proliferation in mammals (Hagiwara *et al.*, 1995). In contrast, CNP is released from the heart and has potent vasodilatory effects *in vitro* in spiny dogfish, *Squalus acanthias* (Evans *et al.*, 1993). In bullfrogs, cardiovascular effects of homologous frog ANP (fANP) or fCNPs have not been examined, except for fBNP exhibited vasodepressor (Fukuzawa *et al.*, 1996). Although chick rectum relaxant activity and hypotensive activity in the rat were observed in the previous pharmacological studies of frog NPs (Sakata *et al.*, 1988; Yoshihara *et al.*, 1990), there are few studies for investigating the physiological roles and comparing the biological activities of three types of homologous NPs (fNPs) in the bullfrog.

In the present study, cardiovascular effects of homologous fANP, fBNP, fCNP I and fCNP II were tested *in vitro* and compared under the same experimental conditions. Since various hormones and nerves have been known to influence the regulation of the cardiovascular system *in vivo* in amphibians (Herman, 1992; West and van Vliet, 1992), we used isolated preparations to eliminate these influences.

MATERIALS AND METHODS

Animals

Male bullfrogs, *Rana catesbeiana*, weighing 250-400 g, were purchased from a supplier of Misato City, Saitama Prefecture and maintained at 20–22°C with a 14L:10D photoperiod. The bullfrogs were fed with raw fish twice a week until 48 hr before experiments.

Vascular experiments

Each bullfrog was doubly pithed and four blood vessels (dorsal

* Corresponding author: Tel. +81-764-45-6633; FAX. +81-764-45-6641.

aorta, iliac artery, femoral artery and femoral vein) were excised and immersed in cooled an aerated frog Ringer solution. Each preparation of blood vessel (7 mm length) was cut helically and suspended between two silk threads, one fixed to a chamber and the other attached to a force-displacement transducer (SB-1T, Nihon Koden Kogyo Co. Ltd., Tokyo, Japan) in a Magnus-type chamber containing frog Ringer solution. The composition of the frog Ringer solution used in all experiments was in mM: NaCl 111, KCl 3.35, $CaCl_2$ 2.7, $NaHCO_3$ 2.38, and glucose 5.5 (all from Wako Pure Chemical Ind., Tokyo, Japan). The solution was prepared daily and aerated for 30 min and its pH was kept at 7.4 ± 0.1. Vascular smooth muscle contraction and relaxation were recorded with a multipurpose polygraph (RM-45, Nihon Koden Kogyo Co. Ltd., Tokyo, Japan). The solution bathing the vascular preparation was maintained at room temperature (20–25°C) and bubbled with air. In the preliminary experiments, when the preparations were treated with norepinephrine (NE) (2×10^{-8} to 2×10^{-5} M), contractions were concentration-related and dorsal aorta was the most sensitive among the four preparations. Thus, we chose the dorsal aorta and the resting tension of 0.7 g in the following experiments.

Experiment 1: relaxant activity of frog NPs on aortic smooth muscle

Isolated preparations were equilibrated without precontractions for 60 min before administrations of NPs. Frog natriuretic peptides (10^{-12} to 10^{-7} g/ml) were cumulatively administered into the bath. When the cumulative concentration-response determination for a series of one NP was obtained, the organ bath was rinsed with Ringer solution 5 times and the tissue was equilibrated for 45 min. In each preparation of the dorsal aorta, four different NPs were tested at random. Data were shown as a percentage of the relaxant activity compared to the maximal response in the same preparation.

Experiment 2: spasmolytic activity of frog NPs on NE-stimulated contraction

10^{-5} M Norepinephrine (NE) was used to obtain control response. The NE was then washed out and the tension allowed to return to baseline level after the stabilizing periods of 45 min. Then a second dose of NE was administered, the subsequent contractions were observed to be stable by 5 min and one of the fNPs (fANP, fBNP, fCNP I and fCNP II) was cumulatively added to the bath. The other fNPs were tested at random after stabilizing intervals of 45 min. At the end of the experiment, 10^{-5} M NE was added to the chamber and the final tension was checked to ensure that no tachyphylaxis occurred. Data were shown as percentages of the decrease in tension compared with the NE contraction-response.

Cardiac experiments

A heart of pithed bullfrog was quickly excised and the blood was rinsed out with aerated frog Ringer solution. The heart was dissected into the atrium and ventricle in the Ringer solution. Only spontaneously beating preparations were used in the experiment. The preparation was then transferred to a glass chamber of the Magnus type, which contained frog Ringer solution. Each preparation was suspended between two silk threads, one fixed to the chamber and the other attached to an isotonic transducer (Type 4537, NEC San-ei Inst. Ltd., Tokyo, Japan). Cardiac muscle contraction and relaxation were recorded with a recorder (Recti-Horiz-8K, NEC San-ei Inst. Ltd., Japan). The solution bathing the cardiac preparation was maintained at room temperature (20–25°C) and bubbled with air. Frog natriuretic peptides (fCNPs, 10^{-10} to 10^{-7} g/ml; fANP and fBNP, 10^{-10} and 10^{-7} g/ml) were added to the bath. When the cumulative concentration-response determination of a series for one NP was obtained, the organ bath was rinsed with the Ringer solution 5 times and the tissue was equilibrated for 15 min. Then the next series of the remaining NPs were examined. Data were shown as a percentage of decrease in tension in comparison with the contraction response. In a preliminary study, –100% contraction of the atrium and ventricle were induced by 10^{-5} and 10^{-4} M acetylcholine, respectively.

Chemicals

Norepinephrine (L-Norepinephrine, NE) and acetylcholine (acetylcholine chloride, ACh) (Wako Pure Chemical Ind., Tokyo, Japan) were dissolved in distilled water. Bullfrog ANP (fANP-24) and frog CNP I (fCNP) were purchased from Peninsula lab. Inc., Calif., USA. Frog BNP (fBNP) and frog CNP II (fCNP II) were prepared at Peptide Inst. Osaka, Japan. Frog ANP, fBNP, fCNP I and fCNP II were also dissolved in distilled water and added in small volumes directly to the incubation chamber.

Statistics

Data are expressed as the mean ± standard error of the mean (SEM). Statistical analysis was performed by one way analysis of variance (ANOVA) with Bonferroni test. Statistical significance was determined at $P< 0.05$.

RESULTS

Effects on vascular muscles

As shown in Fig. 1, frog ANP, fBNP, fCNP I and fCNP II produced a significant, and concentration-dependent, reduction in tension (relaxant effect) in the isolated dorsal aorta. Frog CNP II exhibited the highest activity in vasorelaxation, while fBNP and fCNP I had lower activity than fANP. The EC_{50} of fANP, fBNP, fCNP I and fCNP II were 27.1, 125, 125 and 12.5 ng/ml, respectively. The relative potencies of fBNP, fCNP I and fCNP II to fANP were 0.22, 0.22 and 2.17 respectively,

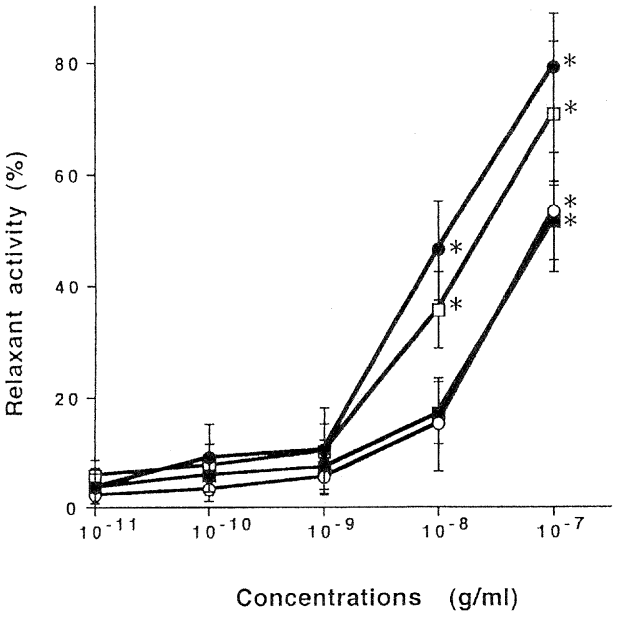

Fig. 1. Vasorelaxant activities of frog ANP (□), frog BNP (■), frog CNP I (○) and frog CNP II (●) on isolated vascular smooth muscle of the systemic artery in the bullfrog. Cumulative concentration-response curves obtained from five experiments (n=5). The percentage relaxation was calculated from the maximal response (100%) induced by frog natriuretic peptides in the same preparation. The results are expressed as mean ± SEM. *Significant differences ($P < 0.05$) compared with the pretreated control levels (0%) of each natriuretic peptide.

when expressed as reciprocal ratios of the EC_{50} values.

Norepinephrine (10^{-5} M) induced the first stage of a steep increase of contraction (tension; 200–300 mg) for 2–5 min and then a long lasting second stage of stable contraction (tension; 150-200 mg) followed for over 40 min until washing out. After contraction of the strips with NE, fNPs were added cumulatively to examine the spasmolytic effects of fNPs at the second stage of contraction. Frog NPs inhibited the NE induced contraction and fCNP II was more potent than fANP and fCNP I (Fig. 2). The IC_{50} values of fANP, fBNP, fCNP I and fCNP II were 92.7, 24.6, 75.1 and 24.7 ng/ml, respectively. The relative potencies of fBNP, fCNP I and fCNP II to fANP were 3.8, 1.2 and 3.8, respectively.

Effects on cardiac muscles

The results are shown in Figs. 3 and 4. In isolated preparations of atrium and ventricle, fCNP I and fCNP II (10^{-10} to 10^{-7} g/ml) produced a significant, and concentration-dependent, reduction in tension. Frog CNP II is more potent than fCNP I. The atrial preparation showed a higher intrinsic activity and higher affinity than the ventricular one. On the other hand, fANP and fBNP (< 10^{-7} g/ml) did not produce any significant effects in the present experiment. Heart rates in isolated preparations of atrium and ventricle were 40 ± 7 and 32 ± 8 beats/min, respectively. Chronotropic responses of the heart to the NPs administrations were insignificant. However, only when the force of contractions was significantly reduced (the negative inotropism) by fCNP II (100 ng/ml), the atrial heart rate was decreased (the negative chronotropic action).

DISCUSSION

This is the first study to examine the effects of homologous NPs on the bullfrog cardiovascular system. If the bio-

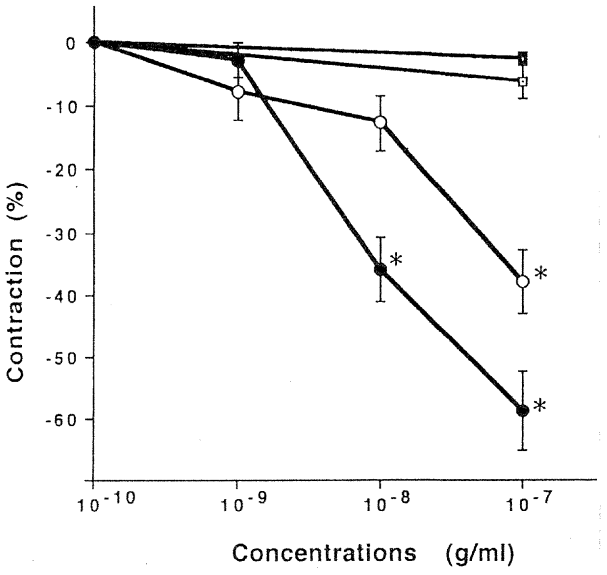

Fig. 3. Negative inotropic activities of frog ANP (□), frog BNP (■), frog CNP I (○) and frog CNP II (●) on isolated atrial preparations in the bullfrog heart. The percentage decrease of isotonic contraction was calculated in relation to the systolic (0%) and diastolic (–100%) contractions. The results are expressed as mean ± SEM (n=5). *Significant differences ($P < 0.05$) compared with the pretreated control levels (0%) of each hormone.

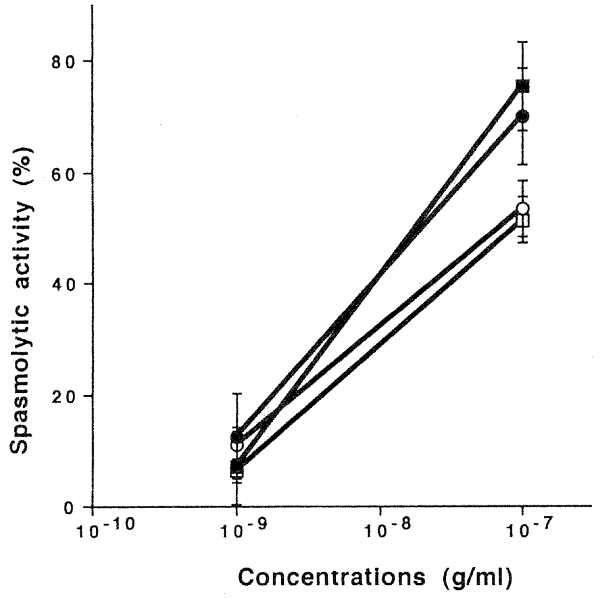

Fig. 2. Spasmolytic activities of frog ANP (□), frog BNP (■), frog CNP I (○) and frog CNP II (●) on the contraction induced by norepinephrine in the systemic artery of the bullfrog. The percentage relaxation obtained by frog natriuretic peptides was calculated in relation to the maximal contraction (100%) after treatment with norepinephrine (10^{-5} M). The results are expressed as mean ± SEM (n=6).

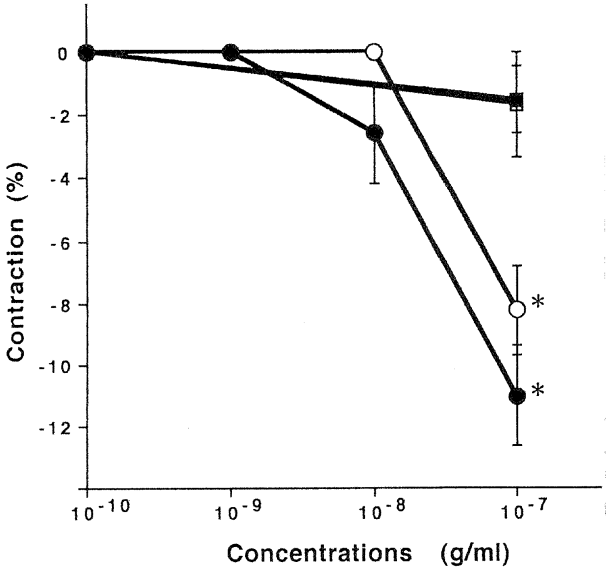

Fig. 4. Negative inotropic activities of frog ANP (□), frog BNP (■), frog CNP I (○) and frog CNP II (●) on isolated ventricular preparations in the bullfrog heart. The percentage decrease of isotonic contraction was calculated in relation to the systolic (0%) and diastolic (–100%) contractions. The results are expressed as mean ± SEM (n=5). *Significant differences ($P < 0.05$) compared with the pretreated control level (0%) of each natriuretic peptide.

logical actions of NPs decrease of blood pressure in the bullfrog, they will diminish cardiac output, reduce peripheral vascular resistance, and/or decrease intravascular volume. In the present study, homologous NPs have a direct cardiovascular effect and fCNP II was the most potent vasorelaxant peptide in the fNP family, although the potency of fNPs was not consistent in the two different experiments, relaxant and spasmolytic activity of fNPs on aortic smoothe muscle. The function of frog CNP II as a vasorelaxant peptide is in contrast to the apparent role of mammalian CNP as a brain neuropeptide (Sudoh *et al.*, 1990; Minamino *et al.*, 1991). However, it is recently suggested that mammalian CNP, which is found in abundance in the endothelium of blood vessels, may regulate vascular tone in a paracrine manner (Amin *et al.*, 1996). In the bioassay for chick rectum relaxant effect, frog CNP I is 7 times more potent than frog ANP-21 (Yoshihara *et al.*, 1990). Although the relaxant effect of frog CNP II has not been examined, fCNP II was widely distributed in brain and peripheral tissues, such as heart, lung, and stomach and stimulated the cyclic GMP formation at the same level with fCNP I in mammalian CNP receptor (Kojima *et al.*, 1994). Thus, it is suggested that fCNPs are vasorelaxant hormones, which may reduce peripheral vascular resistance, and might be more potent than fANP and fBNP in the bullfrog.

The previous work showed that rat ANP (1-28) (10^{-9} to 10^{-6} M) relaxed precontracted aortic rings, but the hormone had no effect on basal tension (about 1,500 mg) in unstimulated arteries of the toad, *Bufo arenarum* (Peral de Bruno and Coviello, 1992). Homologous BNP was twice as potent as human ANP in decreasing arterial blood pressure in the bullfrog (Fukuzawa *et al.*, 1996). Our present study demonstrated that fNPs induced a relaxant effect even in untreated or precontracted helical preparations of the dorsal aorta in the bullfrog. Thus, it may be implied that fNPs might potently reduce the vascular resistance than the heterologous peptides in the bullfrog.

In the present study using isolated heart preparations, fCNP I and II reduced cardiac contraction. Frog CNP II is more potent than fCNP I, and neither fANP nor fBNP produced any significant effects. Quantitative autoradiographic studies showed the presence of NPs receptors in the various parts of the heart of eels, *Anguilla anguilla* and *Anguilla japonica* (Cerra *et al.*, 1996; Sakaguchi *et al.*, 1996). The NPR-C or clearance receptor is very common in the atrium and the ventricular myocardium and NPR-A and -B are present in endocardium and the bulbar layers of the eel heart. According to the study of gene expression of NPRs in rat myocardial cells, ventricular myocyte produced predominantly NPR-A and cardiac fibroblasts synthesize functionally large amounts of both NPR-A and NPR-B (Lin *et al.*, 1995). The present study implies that fCNPs may diminish cardiac output and the NPR-B which specially bind to CNP may be principally present on cardiac muscles of the atrium and ventricles of the bullfrog. However, further studies are necessary to demonstrate directly whether the myocardial cells itself are a site of action for fCNPs in the bullfrog.

Positive chronotropic effects of CNP, but not ANP, were recently described for *in vivo* and *in vitro* sinus node of dog heart (Beaulieu *et al.*, 1996). However, chronotropic responses of the heart to the NPs administrations were insignificant in the bullfrog. When the force of contractions significantly reduced (the negative inotropism) by fCNP II, the atrial heart rates sometimes decreased (the negative chronotropic action). Thus, there may be few fCNP receptor in the impulse conducting system of heart. However, mechanisms of the negative chronotropic effect of fCNP are still unclear in the bullfrog.

The present findings support the conclusion that homologous natriuretic peptides, fANP, fBNP and fCNPs, play a role in the control of cardiovascular system in the bullfrog. Frog CNPs are most potent among fNPs in relation to reduce the cardiac output. Further studies are required to elucidate the presence of fNPs receptors in myocardial cells and the mechanism of action of fCNPs on the heart conduction system.

ACKNOWLEDGMENTS

We thank Dr. Tadao Okamura of Nippon Medical School for his help with the Magnus-type chamber. This study was supported in part by the Tamura Foundation for promotion of Science and Technology, and a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan to MU.

REFERENCES

Amin J, Carretero OA, Ito S (1996) Mechanisms of action of atrial natriuretic factor and C-type natriuretic peptide. Hypertension 27: 684-687

Beaulieu P, Cardinal R, De Lean A, Lambert C (1996) Direct chronotropic effects of atrial and C-type natriuretic peptides in anaesthetized dogs. Br J Pharm 118: 1790–1796

Brenner BM, Ballermann BJ, Gunning ME, Zeidel ML(1990) Diverse biological actions of atrial natriuretic peptide. Physiol Rev 3: 665–699

Cerra MC, Canonaco M, Takei Y, Tota B (1996) Characterization of natriuretic peptide binding sites in the heart of the eel, *Anguilla anguilla*. J Exp Zool 275: 27–35

de Bold AJ, Borenstein HB, Veress AT, Sonnenberg H (1981) A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. Life Sci 28: 89–94

Evans DH, Toop T, Donald J, Forrest Jr JN (1993) C-type natriuretic peptides are potent dilators of shark vascular smooth muscle. J Exp Zool 265: 84–87

Fukuzawa A, Watanabe TX, Itahara Y, Nakajima K, Yoshizawa-Kumagaye K, Takei Y (1996) B-type natriuretic peptide isolated from frog cardiac ventricles. Biochem Biophys Res Commun 222: 323–329

Hagiwara H, Hirose S, Takei Y (1995) Natriuretic peptides and their receptors. Zool Sci 12: 141–149

Herman CA (1992) 3 Endocrinology. In "Environmental Physiology of the Amphibians" Ed by Feder ME, Burggren WW, Univ Chicago Press, Chicago, pp 40–54

Kojima M, Ohyama Y, Miyamoto K, Minamino N, Kangawa K, Matsuo H (1994) Cloning and characterization of a novel natriuretic peptide in frog (*Rana catesbeiana*). J Biol Chem 269: 13136–13140

Koller KJ, Lowe DG, Bennett GL, Minamino N, Kangawa K, Matsuo H, Goeddel DV (1991) Selective activation of the B natriuretic peptide receptor by C-type natriuretic peptide (CNP). Science

252: 120–123
Lin X, Haenze J, Heese F, Sodmann R, Lang RE (1995) Gene expression of natriuretic peptide receptors in myocardial cells. Circulation Research 77: 750–758
Minamino NY, Makino Y, Tateyama H, Kangawa K, Matsuo H (1991) Characterization of immunoreactive human C-type natriuretic peptide in brain and heart. Biochem Biophys Res Commun 179: 535–542
Peral de Bruno M, Coviello, A (1992) Effects of atrial natriuretic peptide and toad heart extract on isolated toad *Bufo arenarum* aortic rings. Gen Comp Endocrinol 88: 424–433
Sakaguchi H, Suzuki H, Hagiwara H, Kaiya H, Takei Y, Ito M, Shibabe S, Hirose S (1996) Whole body autoradiography and microautoradiography in eels after intra-arterial administration of ^{125}I-labeled eel ANP. Am J Physiol 271: R926–R935
Sakata JI, Kangawa K, Matsuo H (1988) Identification of new atrial natriuretic peptides in frog heart. Biochem Biophys Res Commun 155: 1338–1345
Sudoh T, Minamino N, Kangawa N, Matsuo H (1990) C-type natriuretic peptide (CNP): A new member of natriuretic peptide family identified in porcine brain. Biochem Biophys Res Commun 168: 863–870
West NH, van Vliet BN (1992) 7 Sensory mechanisms regulating the cardiovascular and respiratory systems. In "Environmental Physiology of the Amphibians" Ed by Feder ME, Burggren WW, Univ Chicago Press, Chicago, pp 151–182
Winquist RJ, Hintze TH (1990) Mechanisms of atrial natriuretic factor-induced vasodilation. Pharmacol Ther 48: 417–426
Yoshihara A, Kozawa H, Minamino N, Kangawa K, Matsuo H (1990) Isolation and sequence determination of frog C-type natriuretic peptide. Biochem Biophys Res Commun 173: 591–598

(Received April 30, 1997 / Accepted June 19, 1997)