Korean J. of Pharmacology
Vol. 32, No. 1, pp. 51~56, 1996

The Vasodilating Mechanism of Atrial Natriuretic Peptide in 2-kidney 1 Clip Renovascular Hypertensive Rats[1]

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ABSTRACT

The objectives of this study is to find out mechanism of vasodilating effects of ANP in 2K-1C renovascular hypertensive rat aorta and to compare with those of normotensive rat aorta.

In 2K-1C renovascular hypertensive rat, average arterial blood pressure and plasma renin activity were higher than in normotensive rat.

In 2K-1C renovascular hypertensive rat aorta, NE sensitivity was more increased and maximal contraction of aorta by NE was higher than those of normotensive rat aorta.

ANP inhibited NE-induced contraction in both 2K-1C renovascular hypertensive and normotensive rat aorta, concentration-dependently. However, ANP was less effective for relaxing NE-induced contraction in 2K-1C renovascular hypertensive rat aorta than in normotensive rat aorta. ANP inhibited $^{45}Ca^{2+}$ uptake induced by NE in both 2K-1C renovascular hypertensive and normotensive rat aorta.

From these results, inhibition of Ca^{2+} influx may be one of the vasodilating mechanism of ANP in 2K-1C renovascular hypertensive rat aorta. Although the potency of ANP in relaxing NE-induced contractions was attenuated, the efficacy of ANP was not changed in 2K-1C renovascular hypertensive rat aorta compared with that of ANP in normotensive rat aorta.

Abbreviations: ANP, Atrial natriuretic peptide; 2K-1C, 2-kidney 1 clip; NE, norepinephrine; SHR, Spontaneously hypertensive rat; DOC, Deoxycorticosterone; EDTA, Ethylenediaminetetraacetic acid; PSS, Physiological salt solution; TRIS, tris(hydroxymethyl) aminomethane

Key Words: Atrial natriuretic peptide, 2-kidney 1 clip renovascular hypertensive rat, Ca^{2+} movement

INTRODUCTION

ANP is biosynthesized and released from atrium of heart in response to auricular stretch (De Bold *et al.*, 1981; Cantin and Genest, 1985). ANP has many biological effects including natriuretic action resulting in reducing of blood circulation volume (Brenner *et al.*, 1990), inhibition of aldosterone, renin and vasopressin release (Lee *et al.*, 1987; Rosenzweig and Seidman, 1991). Besides, ANP is found in central nervous system (Tanaka *et al.*, 1984) and regulates the drinking appetite (Samson, 1985).

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[1]This paper was supported by research fund offered by Chungbuk National University Academic Research Foundation, 1993.

In various animal models of hypertension, including the SHR, one and two kidney and DOC-salt hypertension, ANP is an effective blood pressure lowering agent (Seymour *et al.*, 1985). So, ANP is believed to be involved in arterial blood pressure regulation and electrolyte and water balance.

In smooth muscle, ANP is known to activate particulate guanylyl cyclase, which results in cGMP formation (Winquist *et al.*, 1984). Furthermore, ANP inhibits Ca^{2+} influx and intracellular Ca^{2+} release (Chiu *et al.*, 1986). Because 2-kidney 1 clip renovascular hypertensive rat is a good model for study of human renal hypertension, we attempted to investigate the vasorelaxing mechanism(s) of ANP on 2K-1C renovascular hypertensive rat aorta and to compare them with those of ANP in normotensive rat aorta.

MATERIALS AND METHODS

Preparations

Male Sprague-Dawley rats, weighing 150 g were made hypertensive by placing a silver clip (0.25 mm gap) around the left renal artery (2-kidney 1 clip). Blood pressures were measured at 2 weeks after the operative intervention using carotid catheterization.

Recordings of mechanical activity

After measurement of blood pressures, animals were killed and thoracic aortas free of excess connective tissue were excised. Endothelium was removed by gently rubbing the intimal space with a cotton swab. Segments 2 cm long were placed in organ chambers containing 20 ml of normal physiological salt solutions (PSS) of the following composition (in mM): NaCl 136.9, KCl 5.4, glucose 5.5, $NaHCO_3$ 23.8, $CaCl_2$ 1.5, $MgCl_2$ 1.0 and EDTA 0.01. Isosmotic 65.4 mM K^+PSS was made by substituting 60 mM NaCl in the normal PSS with equimolar KCl.

Muscle tension was recorded isometrically with force-displacement transducer connecting to the polygraph (Gould). Passive tension of 1 g was initially applied and muscle strips were allowed to equilibrate at 37℃ for 60 min and aerated with 95% O_2 and 5% CO_2. ANP (rat atriopeptin III, Peninsula) was cumulatively applied when the contractile tension induced by stimulants reached a steady level. The concentration of ANP required to induce a 50% inhibition of contraction (IC_{50}) was calculated from the cumulative concentration-inhibition curves.

Measurements of Ca^{2+} uptake

Muscle strips were allowed to equilibrate for 2 hrs in normal PSS and then incubated with [$^{45}Ca^{2+}$] (2 μCi/ml, New England Nuclear) for 5 min. ANP was added 30 min before the [$^{45}Ca^{2+}$] exposure. NE was added simultaneously with [$^{45}Ca^{2+}$]. Muscle strips were then washed to remove extracellular [$^{45}Ca^{2+}$] for 30 min in an ice-cold lanthanum solution $LaCl_3$ 73.8 mM, glucose 5.5 mM, and TRIS 24.0 mM. This solution was adjusted to pH 6.8~6.9 at 0.5℃ with 1 N maleic acid. After the La^{3+}-wash period, muscle strips were removed from holders, blotted, placed in scintillation vials and [$^{45}Ca^{2+}$] was extracted overnight with 1 ml of 20 ml EGTA solution. Scintillation cocktail (Beckman) was added to each vial and radioactivity was counted with a liquid scintillation counter (Beckman).

Measurements of plasma renin activity

Plasma renin activity was measured by detection of angiotensin I production using radioimmunoassay (Cho *et al.*, 1987). Briefly, aliquots (420 μl) of rat plasma were added to 50 μl of maleate buffer (pH 5.88, 1.5 M), 10 μl of each 8-hydroxyquinoline (3.4 mM), neomycin (0.4%) and phenylmethylsulfonylfluoride (7.5 mM). Aliquots (50 μl) of this mixture were taken for the determination of basal angiotensin I levels. The remaining mixture was then incubated at 37℃ for 120 min. The angiotensin I generated was measured by the method of Harber *et al.*, 1969.

Drugs

Atrial natriuretic peptide (rat atriopeptin III, 5~28, Peninsula laboratories), Norepinephrine bitartrate (Sigma), EGTA (Sigma), TRIS (Sigma), Heparin (Sigma) and [$^{45}Ca^{2+}$]Cl_2, All other chemicals were of reagent grade purity.

Statistics

Group mean values were compared using unpaired two-tailed Students t test. A P value of <0.05 was taken as significant.

RESULTS

Blood pressures and plasma renin activity

The mean arterial blood pressure of sham-operated controls was 102 ± 5 mmHg (n=8) and that of 2K-1C renovascular hypertensive rats was 167 ± 12 mmHg (n=8). Plasma renin activity of sham-operated controls was 3.7 ± 0.3 ng angiotensin I/ml/hr (n=14) and that of 2K-1C renovascular hypertensive rats was 6.0 ± 1.3 ng angiotensin I/ml/hr (n=14) (Table 1).

NE sensitivity

Concentration-response curve of NE is shown in Fig. 1. In control aorta, 10^{-7} M NE developed 0.68 ± 0.07 g and 0.99 ± 0.09 g in 2K-1C renovascular hypertensive rat aorta, individually. In control aorta, EC_{50} was 1.7×10^{-9} M NE. In 2K-1C renovascular hypertensive rat aorta concentration-response curve was shifted to the left and EC_{50} was decreased to 7.5×10^{-10} M NE. Furthermore, the maximal contractions of NE were increased more in 2K-1C renovascular hypertensive rat aorta than in sham-operated control.

Inhibitory effect of ANP on tension

The inhibitory effect of ANP on 3×10^{-8} M

Table 1. Blood pressure and plasma renin activity of sham-operated normotensive rat and 2K-1C renovascular hypertensive rat

	Blood pressure (mmHg, n=8)	Plasma renin activity (ng angiotensin I/ml/h, n=14)
Normotensive rat	102 ± 5	3.7 ± 0.3
2K-1C hypertensive rat	167 ± 12*	6.0 ± 1.3

*Significantly different from normotensive rat blood pressure($P<0.01$)

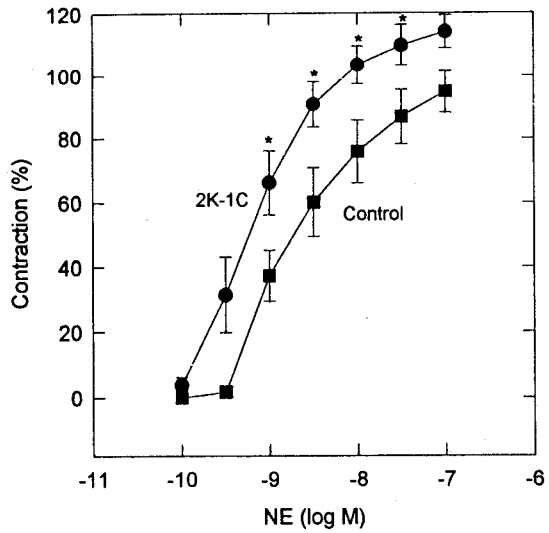

Fig. 1. Comparisons of the norepinephrine (NE)-induced contraction between in the 2K-1C renovascular hypertensive rat aorta (●, n=8) and in normotensive rat aorta (■, n=8), 100 % means the magnitude of high-K^+-induced contractions in rat aortas. *indicates P values were less than 0.05 versus control.

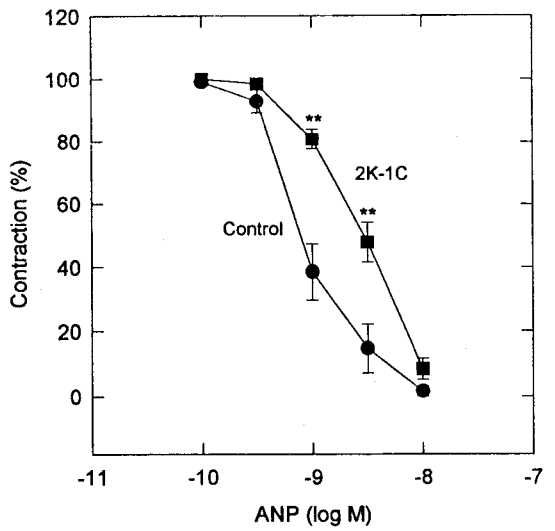

Fig. 2. Inhibition curves of 3 X 10^{-8} M NE-induced contraction by ANP in 2K-1C renovascular hypertensive (■, n=8) and normotensive rat aortas (●, n=8). ** indicates P values were less than 0.01 versus control.

NE-induced contractions was investigated. ANP, concentration-dependently, inhibited the NE-induced contractions of 2K-1C renovascular hypertensive rat aorta less potently than those of sham-operated control. IC_{50} of ANP was 7.8×10^{-10} M in control aorta and IC_{50} of ANP was increased to 2.8×10^{-9} M in 2K-1C renovascular hypertensive rat aorta (Fig. 2). However, the maximal concentrations of ANP (10^{-8} M) decreased the NE-induced contractions to the basal level in both aortas.

Inhibitory effect of ANP on Ca^{2+} movement

In control aorta, resting $^{45}Ca^{2+}$ uptake was 92.0 ±3.8 nmol/g wet wt/5 min and 3×10^{-8} M NE increased $^{45}Ca^{2+}$ uptake to 126.6±7.2 nmol/g wet wt/5 min. Pretreatment of 10^{-8} M ANP for 30 min reduced NE-induced $^{45}Ca^{2+}$ uptake to 95.3± 7.7 nmol/g wet wt/5 min. In 2K-1C renovascular hypertensive rat aorta, resting $^{45}Ca^{2+}$ uptake was 101.3±6.5 nmol/g wet wt/5 min and it was increased to 128.6±7.8 nmol/g wet wt/5 min by 3×10^{-8} M NE. Pretreatment of 10^{-8} M ANP for 30 min reduced NE-induced $^{45}Ca^{2+}$ uptake to 107.8±3.8 nmol/g wet wt/5 min in 2K-1C renovascular hypertensive rat aorta (Fig. 3).

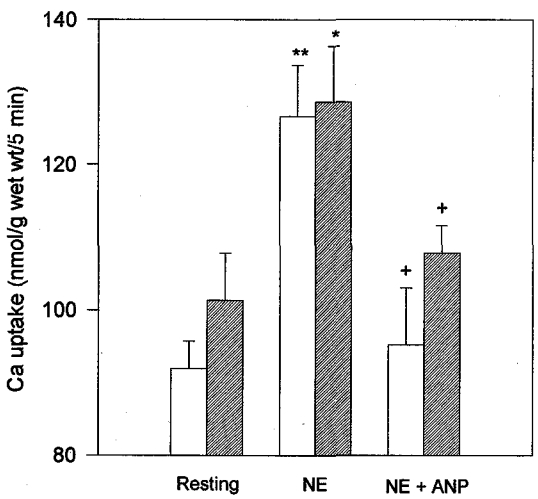

Fig. 3. Bar graph showing the effect of 10^{-8} M ANP on the 3×10^{-8} M NE-induced $^{45}Ca^{2+}$ uptake in 2K-1C renovascular hypertensive (filled bars) and normotensive rat aortas (open bars). *and **indicates P values less than 0.05 and 0.01 versus resting, individually. +indicates P values less than 0.05 versus NE, individually. All values are the mean±SE for six to eight determinations.

DISCUSSION

The goal of this study is to find out the vasodilating mechanism of ANP in 2K-1C renovascular hypertensive rat aorta and to compare it with those of ANP in normotensive control aorta.

In 2K-1C renovascular hypertensive rat, mean arterial pressure and plasma renin activity was raised 2 weeks after operation. As shown in Fig. 1, NE increased contractile tension concentration-dependently in control aorta. In 2K-1C renovascular hypertensive rat aorta, concentration-response curve was shifted to the left and EC_{50} was decreased to 2.2 folds. Therefore, NE sensitivity seems to be increased in 2K-1C renovascular hypertensive rat aorta. It has been known that renal hypertension impairs the formation of endothelium-derived relaxing factors (NO) (Dohi *et al.*, 1991). In this study, it is not clear whether the impaired formation of NO resulted in increase in NE sensitivity or not in 2K-1C renovascular hypertensive rat aorta.

In Fig. 2, ANP , concentration-dependently, depressed NE-induced contractions in both aorta. However, the dilating curve of ANP in 2K-1C renovascular hypertensive rat aorta was shifted to the right in comparison to the control aorta. IC_{50} of ANP in 2K-1C renovascular hypertensive rat aorta was increased 3.6 folds compared with that of ANP in control aorta. So, the potency of ANP is decreased in 2K-1C renovascular hypertensive rat aorta in comparison to normotensive control aorta. On the other hand, the maximal concentrations of ANP reduced NE-induced contractions to the basal level in both aorta. This means the dilating efficacy of ANP on the NE-induced contraction in 2K-1C renovascular hypertensive aorta was not changed compared with that of ANP in normotensive control aorta.

The contraction of smooth muscle tissues is associated with an increase in cytosolic Ca^{2+}

and the Ca^{2+}-dependent phosphorylation of the 20-kDa myosin light chain by activated myosin light chain kinase (Dillon *et al.*, 1981). To investigate one of the vasodilating mechanism of ANP on the NE-induced contractions in 2K-1C renovascular hypertensive rat aorta, we measured $^{45}Ca^{2+}$ uptake. Resting $^{45}Ca^{2+}$ uptake was not statistically different between two rat aortas. In normotensive control aorta, NE increased $^{45}Ca^{2+}$ uptake to 1.4 folds from resting values. ANP inhibited NE-increased $^{45}Ca^{2+}$ uptake to the basal level. In 2K-1C renovascular hypertensive aorta, NE also increased $^{45}Ca^{2+}$ uptake to 1.3 folds from resting values. The increased ratios of $^{45}Ca^{2+}$ uptake by NE was not different between two rat aortas. ANP inhibited NE-increased $^{45}Ca^{2+}$ uptake to the basal level in 2K-1C renovascular hypertensive rat aorta. Therefore, inhibition of Ca^{2+} uptake may be one of the vasodilating mechanisms of ANP on the NE-induced contractions in 2K-1C renovascular hypertensive rat aorta and in normotensive rat aorta.

In deoxycorticosterone acetate salt hypertensive rats, inositol phosphate production after stimulation with endothelin was lower versus control rats (Fluckiger *et al.*, 1992). It was reported that α-human atrial natriuretic peptide inhibited NE-induced synthesis of myo-inositol 1,4,5-triphosphate in rabbit aorta (Kajikuri and Kuriyama, 1990). The effect of ANP on inositol metabolism by NE in 2K-1C renovascular hypertensive rat aorta is uncertain at present and should be clarified in further study.

In conclusion, 2K-1C renovascular hypertensive rat aorta showed that NE sensitivity and maximum contractions of NE were increased in comparison to normotensive control aorta. ANP reduced NE-induced contractions in both aortas. Although the vasodilating potency of ANP on NE-induced contractions were decreased in 2K-1C renovascular hypertensive rat aorta, the vasodilating efficacy of ANP was not changed compared with that of ANP in normotensive control aorta. The inhibition of Ca^{2+} entry may be one of the vasodilating mechanism of ANP on NE-induced contractions in 2K-1C renovascular hypertensive rat aorta and in normotensive rat aorta.

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=국문초록=

2-kidney 1 clip 신혈관성 고혈압흰쥐에서의 심방이뇨펩타이드의 혈관이완작용의 기전

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정 진 영 · 안 영 철 · 김 헌 식
고 규 영* · 안 희 열 · 김 명 석**

본 연구의 목적은 2-kidney 1 clip (2K-1C) 신혈관성 고혈압흰쥐에서 심방이뇨펩이드의 혈관이완작용의 기전을 규명하고 정상혈압흰쥐에서의 혈관이완작용과 비교하는 것이다. 2K-1C 신혈관성 고혈압흰쥐는 정상혈압흰쥐에 비하여 평균동맥압의 유의한 상승과 혈장레닌활성의 증가가 관찰되었다. 2K-1C 신혈관성 고혈압흰쥐의 대동맥에서 norepinephrine (NE)의 수축력의 감수성 및 최대 수축력이 정상혈압흰쥐의 대동맥보다 증가하였다. 심방이뇨펩타이드는 NE에 의한 수축을 농도-의존적으로 각각의 혈압군에서 억제하였다. 그러나, 2K-1C 신혈관성 고혈압흰쥐에서 심방이뇨펩타이드의 NE 억제 작용은 전체적으로 정상혈압흰쥐에서 보다 감소되었다. 그러나 최대 용량의 심방이뇨펩타이드의 NE 이완작용은 양 고혈압군에서 차이가 없었다. 2K-1C 신혈관성 고혈압흰쥐에서 NE에 의하여 Ca^{2+}의 유입이 유의하게 증가하였고, 심방이뇨펩타이드는 이 증가를 억제하였다. 정상혈압흰쥐에서도 심방이뇨펩타이드는 NE에 의하여 유의하게 증가된 Ca^{2+}의 유입을 억제하였다.

이상과 같은 결과로 볼 때 정상혈압흰쥐와 2K-1C 신혈관성 고혈압흰쥐의 심방이뇨펩타이드의 이완작용의 기전에는 Ca^{2+}의 유입 차단이 관여할 것으로 추측되며, 이때 심방이뇨펩타이드의 NE 수축억제에 대한 potency는 2K-1C 신혈관성 고혈압 흰쥐에서 감소하였으나 efficacy는 정상혈압흰쥐와 비교하여 변함이 없음이 관찰되었다.