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博士學位論文

Direct Vascular Actions of Indapamide in Aorta from Renal Hypertensive Rats

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Thiazide 이뇨제는 이뇨작용과 더불어 혈관 확장효과도 존재함이 알려져 있다. Thiazide 이뇨제가 norepinephrine과 vasopressin에 의한 혈관 수축반응에 미치는 영향을 검토하고 two-kidney, one clip (2K1C) 신성고혈압 모델에서 차이가 있는지 구명하고자 본 연구를 시행하였다.

흰쥐 일측 신동맥에 clip을 장치한 고혈압군과 대조군의 적출 흉부대동맥 표본을 hydrochlorothiazide, indapamide 및 chlorthalidone으로 각각 전처치한 상태에서 norepinephrine과 vasopressin에 의한 수축반응에 미치는 영향을 양군에서 비교 검토하였다.

Norepinephrine과 vasopressin에 의한 수축반응은 indapamide에 의해 2K1C 고혈압군에서 억제되었으나 대조군은 영향받지 않았다. Indapamide에 의한 수축 억제효과는 혈관 내피층 제거시 소실되었다. Hydrochlorothiazide 및 chlorthalidone은 대조군은 물론 2K1C 고혈압군에서 도 norepinephrine과 vasopressin에 의한 수축반응에 영향을 미치지 않았다.

이상의 실험결과는 indapamide가 2K1C 신성고혈압 상태에서 norepinephrine과 vasopressin에 의한 수축반응을 억제시키며 억제효과에 혈관 내피층이 관여함을 시사한다.

I . INTRODUCTION

Thiazide diuretics are efficacious in lowering elevated blood pressure and effective in reducing morbidity and mortality in patients with mild to severe hypertension[1,2]. Despite the fact that thiazide diuretics have been in clinical use for a long time, the mechanism underlying their blood pressure-reducing effect has not yet been fully clarified[3,4]. Hemodynamically, the diuretic effect of thiazides is known to decrease blood pressure primarily by reducing extracellular fluid volume and cardiac output[5]. Diuretics have also been shown to increase both plasma and urinary catecholamines by reflex-type mechanisms, such as sympathetic hyperactivity in response to excessive loss of salt and water. This compensatory sympathetically-mediated effect increases peripheral vascular resistance and would blunt the antihypertensive activity of drug[6]. With long-term thiazide treatment, however, it has been found that the plasma volume returns to baseline values and peripheral resistance decreases, suggesting a direct vascular action in addition to the diuretic effect[7]. Several studies have demonstrated a direct vascular action of the thiazide diuretics, hydrochlorothiazide or methyclothiazide, and the thiazide-like diuretics, indapamide and chlorthalidone[8-10].

Previous studies have shown that methyclothiazide inhibit the contractile response induced by norepinephrine in spontaneously

hypertensive rat aorta, only when the endothelium is present[10,11]. The results imply that the blood pressure lowering activity of methyclothiazide, in addition to the diuretic effect, is mediated by a reduction of the vascular response to the action of vasoconstricting stimuli via an endothelium-dependent mechanism in hypertension. In physiological states, the vascular endothelium plays a critical role in maintaining the vascular tone because vascular smooth muscle is always exposed to many kinds of vasoconstrictor stimuli such as norepinephrine, serotonin and arginine vasopressin. It has been proposed that various forms of hypertension are characterized by a dysfunctional endothelium[12]. We have also observed that the role of endothelium is impaired in two-kidney, one clip (2K1C) renal hypertension[13,14]. Although the inhibitory effect of the thiazide diuretic on the norepinephrine-induced contractile response via vascular endothelial modulation was demonstrated in spontaneous hypertension[10,11], the effect of diuretics on the contractile stimuli in 2K1C hypertension has not been intensively established.

The purpose of the present study was to examine, in vitro, the inhibitory effect of hydrochlorothiazide, indapamide and chlorthalidone on the vascular reactivity to norepinephrine and arginine vasopressin in 2K1C renal hypertensive and sham-clipped normotensive rat isolated aortic ring preparations, and to determine whether the anti-vasoconstrictor effect of these drugs is dependent on intact vascular endothelium.

Ⅱ. METHODS

1. Development of 2K1C hypertension

Under thiopental (40 mg/kg, IP) anesthesia, Male Sprague-Dawley rats (150-200 g) were made hypertensive by constricting the left renal artery with a silver clip having an internal gap of 0.2 mm, resulting in partial occlusion of renal perfusion. The contralateral kidney remained untouched and the wound was closed. A group of age-matched rats received a sham treatment: they were operated as in 2K1C rats, except for that no clipping was made. All animals were fed normal chow and were given tap water. They were used at 10 weeks after the clipping, since the endothelial dysfunction is associated with a duration of hypertension[15]. Hypertensive rats were selected on the basis of the systolic blood pressure measured in a conscious state by use of tail cuff method.

2. Tissue preparation

Rats were killed by stunning and exanguination. Thoracic aortae were rapidly removed and placed in cold physiological salt solution (PSS) of the following composition (mM): NaCl 118.3, KCl 4.7, $NaHCO_3$ 25, $MgCl_2$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5

and glucose 11.1. The vessels were cleaned adventitia and cut into 2~3 mm long cylindrical rings under a dissecting microscope. In some preparations, the vascular endothelium was mechanically removed by rubbing gently with a cotton swab. Successful removal of endothelial cells from aortic rings was confirmed by the inability of acetylcholine to induce relaxation.

The aortic rings were suspended by means of two triangle-shaped stainless steel holders in the vessel lumen in organ chambers containing 15 mL of PSS maintained at 37℃, and bubbled with a mixture of 95% O_2 and 5% CO_2 (pH 7.4) throughout the experiment. One of the holders was fixed at the bottom of the chambers and the other was connected to a force displacement transducer (Grass FTO3) for measurement of isometric tension (Fig. 1). Before initiating specific experimental protocols, the aortic rings were stretched to the point of their optimal length-tension relationship 2 g, determined in similar preliminary experiments using repeated exposure to 60 mM KCl solution (obtained by equimolar replacement of NaCl by KCl in the physiological solution), and allowed to equilibrate during the period of 90 min. After an equilibration period, the rings were maximally contracted by the 60 mM KCl solution to test their contractile capacity.

ISOLATED TISSUE BATH

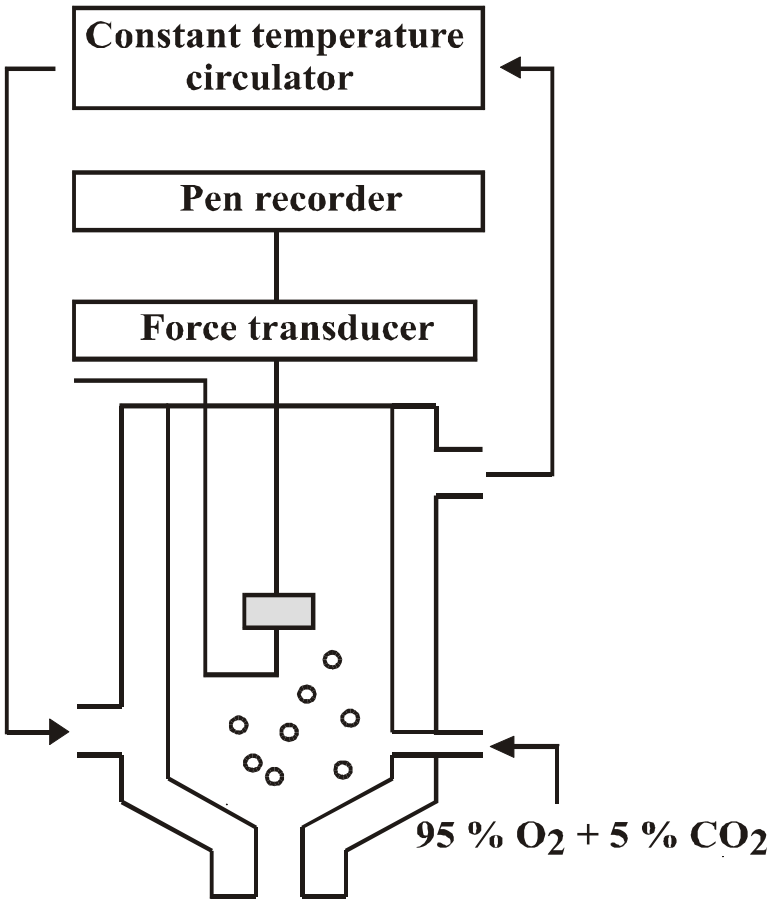

Fig. 1. A schematic representation of the recording system for isometric contraction with 15 mL tissue bath.

3. Experimental Protocols

Aortic rings with or without endothelium were contracted with increasing cumulative concentrations of norepinephrine (10^{-9}~10^{-5} M) and arginine vasopressin (10^{-10}~$10^{-6.5}$ M) to generate concentration-response curves. The response to norepinephrine and arginine vasopressin was examined in parallel rings, which had been incubated for 15 min with either hydrochlorothiazide, indapamide or chlorthalidone and their solvent (control). The diuretic agents were used at concentrations of 10^{-6}, 10^{-5} and 10^{-4} M[11].

To avoid possible time-dependent changes in the responsiveness of the endothelium and vascular smooth muscle, only a single concentration-curve was obtained in each ring preparation.

4. Drugs

The drugs used were norepinephrine bitartrate, acetylcholine hydrochloride, arginine vasopressin, hydrochlorothiazide, indapamide and chlorthalidone. They were purchased from Sigma Chemical Co (St. Louis, MO, USA). Thiazide diuretics were dissolved in dimethylsulfoxide (DMSO) and the other agents were dissolved in distilled water. All drugs were administered in volumes not exceeding 0.5 % of the bath

volume. Final bath concentration of DMSO were less than 0.05 %. At this concentration DMSO did not alter norepinephrine- and arginine vasopressin-induced contractions.

5. Analysis and statistics

Contractions are expressed as a percentage of the contraction developed by the 60 mM KCl. Data are presented as means and standard error of the mean for the number of aortic rings indicated in parentheses. The concentration of an agonist causing half-maximal contraction (EC_{50} value) or IC_{50} are calculated for each experiment and expressed as negative log molar (pD_2). Statistical comparisons were performed by means of unpaired t test or analysis of variance with repeated measurements. Differences were considered to be statistically significant when P value was less than 0.05.

Ⅲ. RESULTS

Ten weeks after the operative intervention, the systolic blood pressure were 134±3 mmHg (n=41) and 196±4 mmHg (n=39) in sham-clipped control and 2K1C hypertensive rats, respectively ($P<0.05$, Fig. 2). The magnitude of KCl (60 mM)-induced isometric tension development was comparable between the two groups (1.37±0.09 g in control; 1.51±0.11 g in 2K1C). The diuretics alone did not alter the basal response in aortic rings from 2K1C and sham rats until a concentration of 10^{-4} M.

1. Effects of hydrochlorothiazide, indapamide or chlorthalidone on norepinephrine-induced contraction

Norepinephrine induced a concentration-dependent contraction of aortic rings from sham-clipped and 2K1C rats. The sensitivity (pD_2) to norepinephrine was increased in aortic rings from 2K1C than in those from sham rats (7.66±0.08 vs 6.91±0.07, $P<0.05$). Indapamide did not induce any significant inhibitory effect on the contractile response to norepinephrine in aortic rings from sham rats. In 2K1C rats, however, the contractile response to norepinephrine was attenuated by indapamide treatment (pD_2: 7.19±0.06, $P<0.05$). The maximal

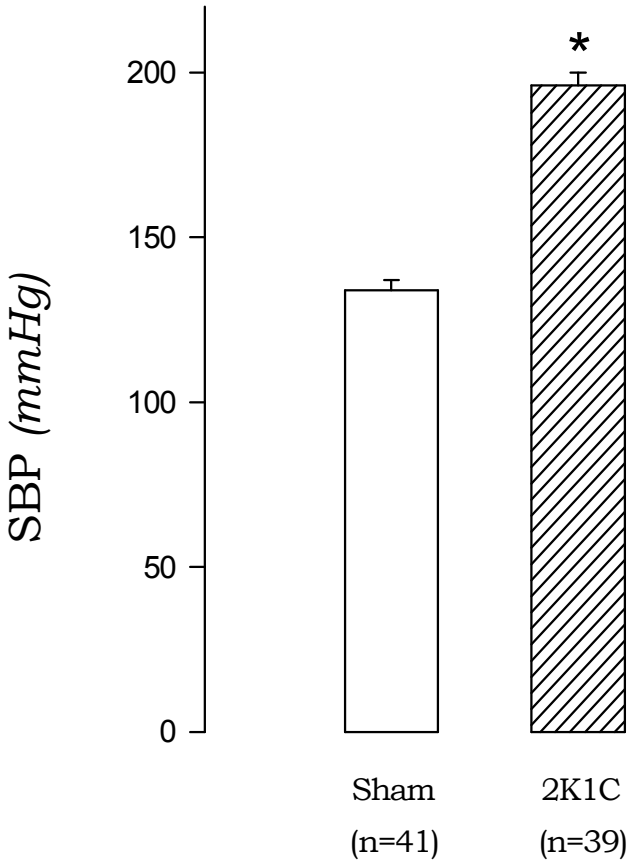

Fig. 2. Systolic blood pressure in 2K1C hypertensive and sham-operated control rats. *P<0.05, compared with the sham value.

response to norepinephrine was inhibited by 23±4.7 % of the control response (Fig. 3, Fig. 4). The inhibitory effect of indapamide on the contractile response to norepinephrine was abolished when the endothelium had been removed (Fig. 5). Hydrochlorothiazide and chlorthalidone affected the contraction to norepinephrine neither in 2K1C nor in sham rat aorta (Fig. 6, Fig. 7, Fig. 8, Fig. 9).

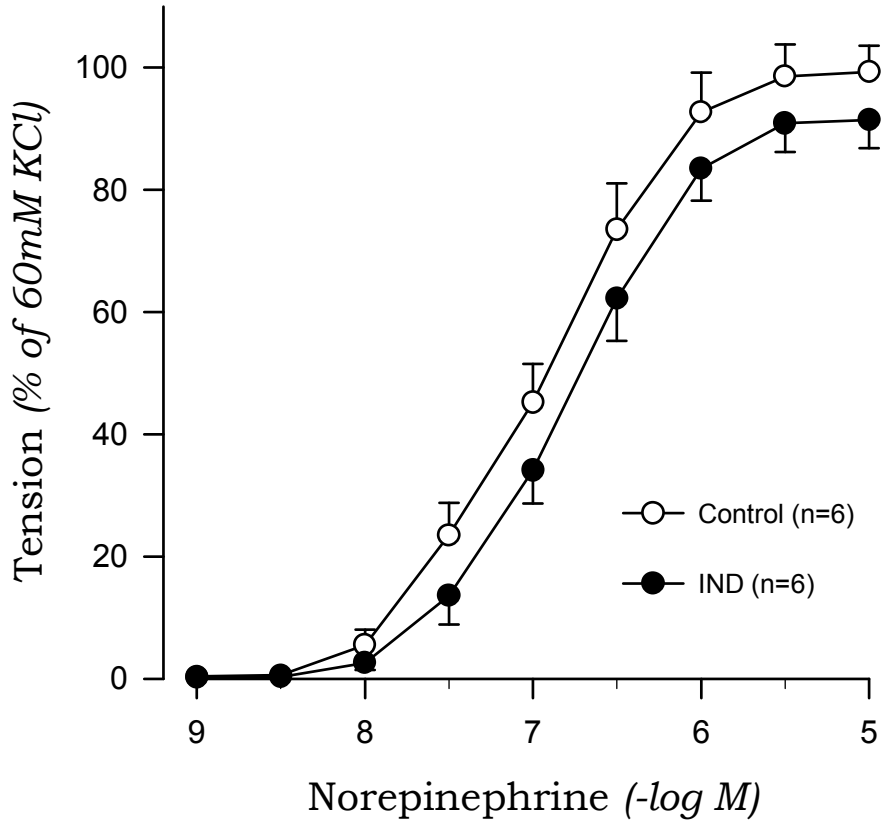

Fig. 3. Effects of indapamide (IND) on contractile responses to norepinephrine in aortic rings from sham-operated rats. Values are mean±SE of n experiments.

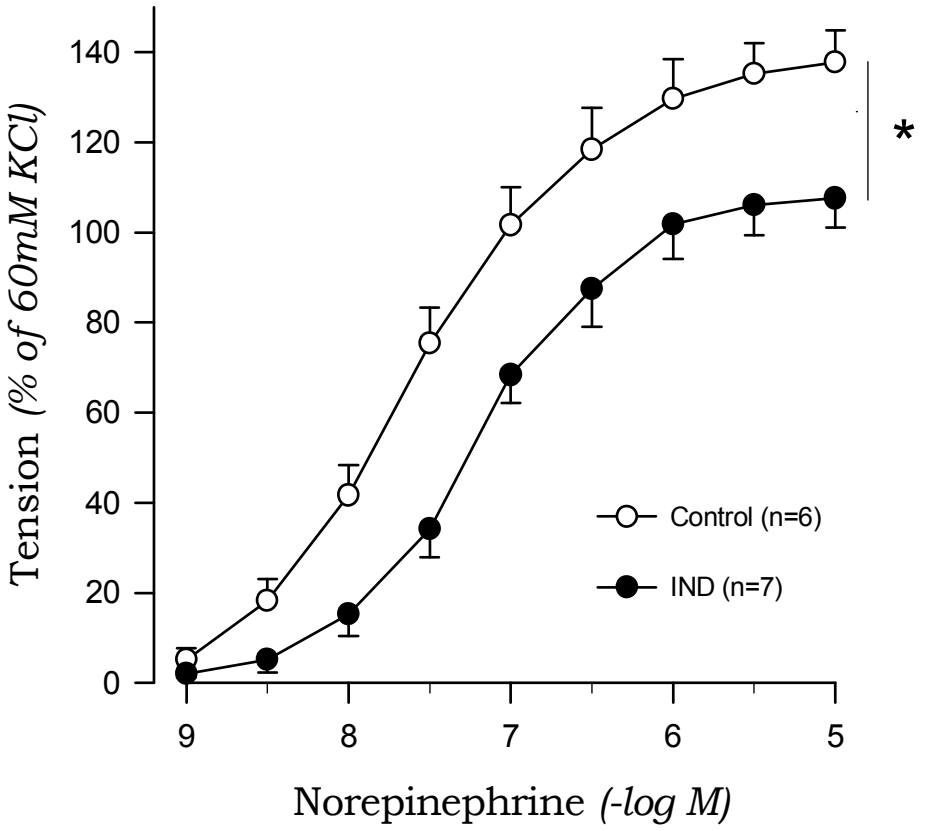

Fig. 4. Effects of indapamide (IND) on contractile responses to norepinephrine in aortic rings from 2K1C hypertensive rats. *P<0.05, compared with the control value.

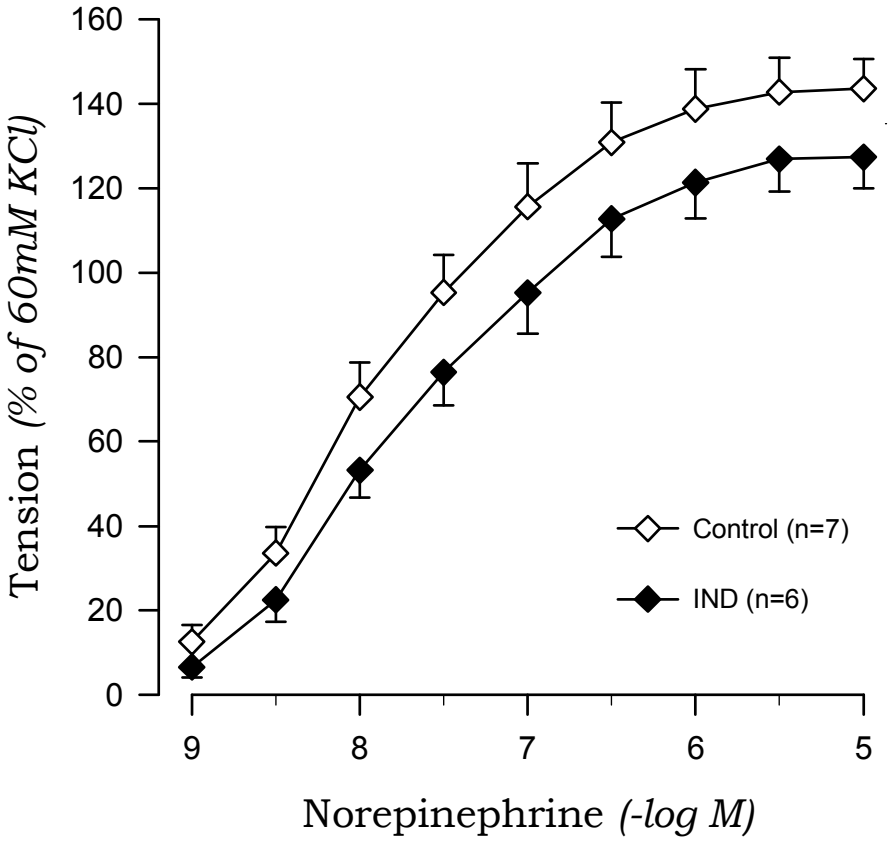

Fig. 5. Effects of indapamide (IND) on contractile responses to norepinephrine in aortic rings without endothelium from 2K1C hypertensive rats.

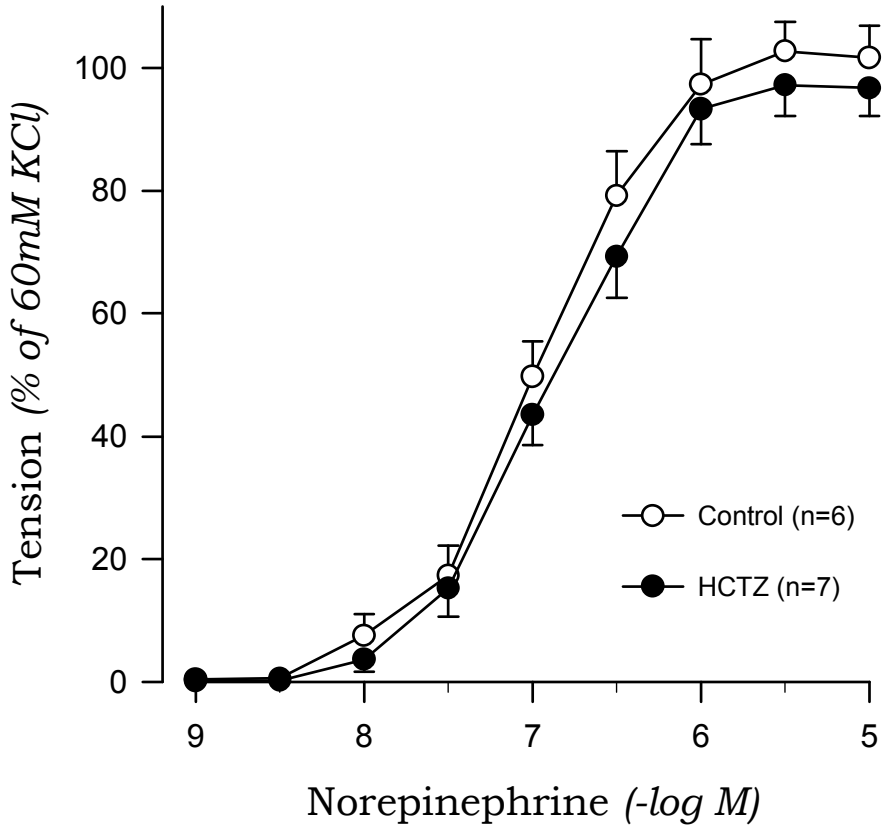

Fig. 6. Effects of hydrochlorothiazide (HCTZ) on contractile responses to norepinephrine in aortic rings from sham-operated rats.

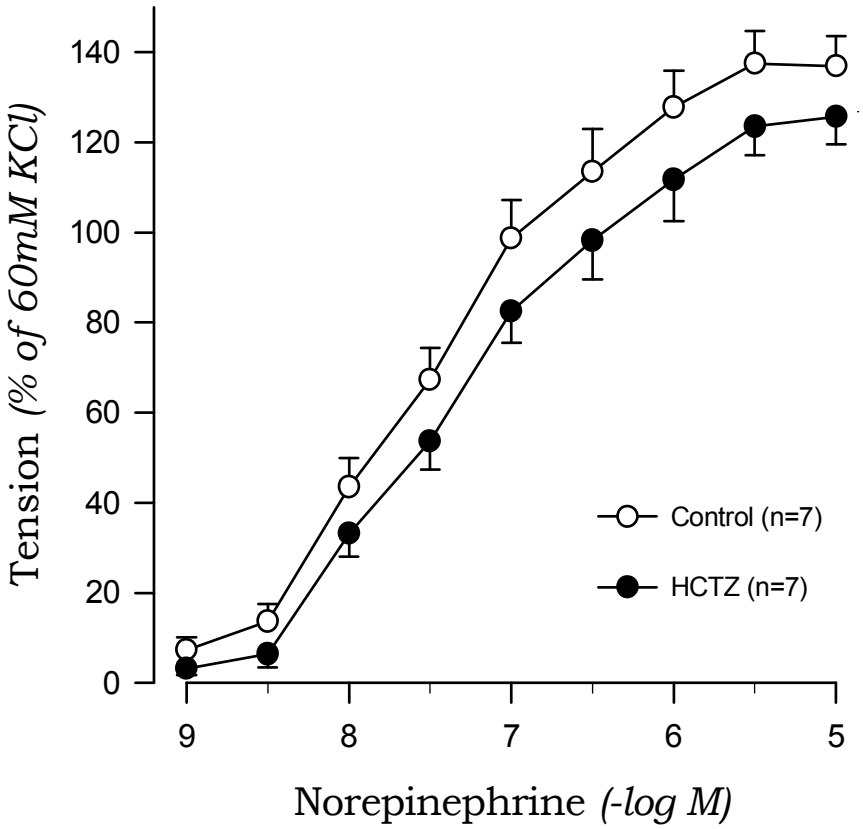

Fig. 7. Effects of hydrochlorothiazide (HCTZ) on contractile responses to norepinephrine in aortic rings from 2K1C hypertensive rats.

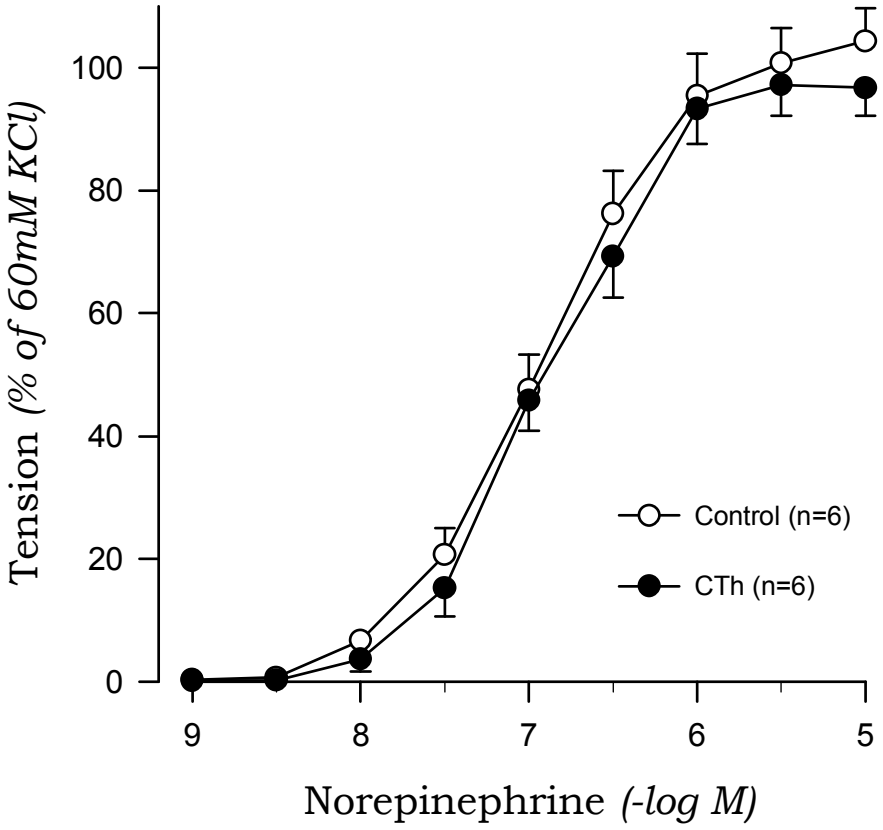

Fig. 8. Effects of chlorthalidone (CTh) on contractile responses to norepinephrine in aortic rings from sham-operated rats.

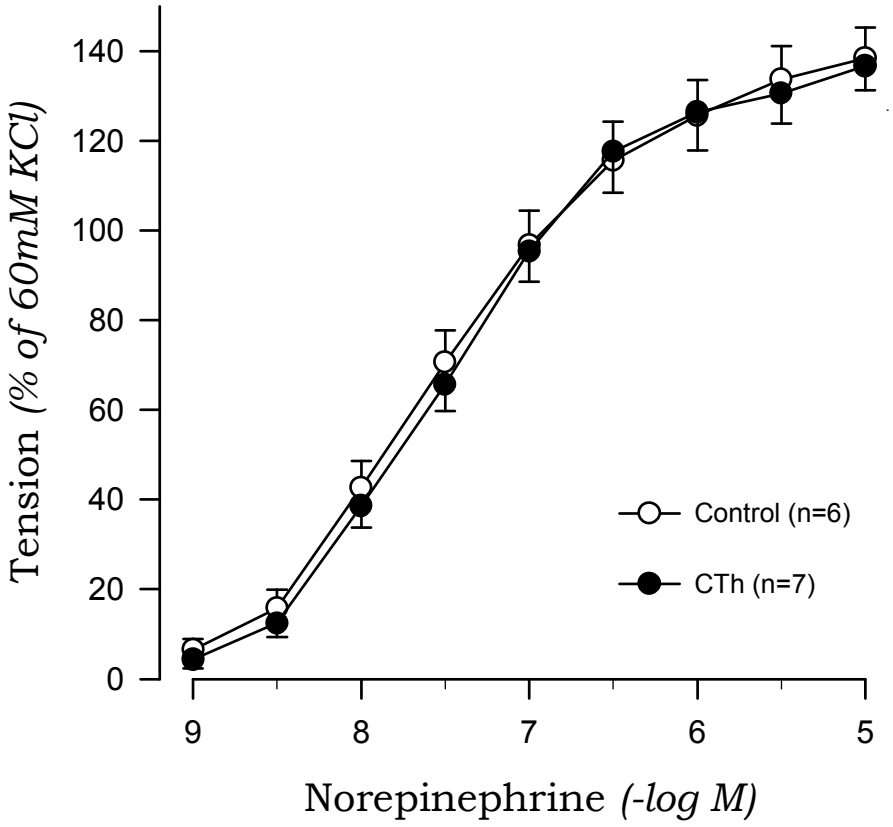

Fig. 9. Effects of chlorthalidone (CTh) on contractile responses to norepinephrine in aortic rings from 2K1C hypertensive rats.

2. Effects of hydrochlorothiazide, indapamide or chlorthalidone on vasopressin-induced contraction

The sensitivity (pD_2) to arginine vasopressin was increased in aortic rings from 2K1C than in those from sham rats (8.67±0.07 vs 8.38±0.06, $P<0.05$). Indapamide attenuated the contractile response induced by vasopressin in aortic rings from 2K1C rats (pD_2: 8.43±0.05, $P<0.05$), of which the maximal response was inhibited by 28±5.3 % of the control response. However, the contractile response to vasopressin was not altered by indapamide pretreatment in sham rats (Fig. 10, Fig. 11). In aortic rings without endothelium of 2K1C rats, the inhibitory effect of indapamide on the contractile response to vasopressin was abolished (Fig. 12). Hydrochlorothiazide and chlorthalidone failed to inhibit the contraction response to vasopressin in aortic rings from 2K1C and sham rats. (Fig. 13, Fig. 14, Fig. 15, Fig. 16).

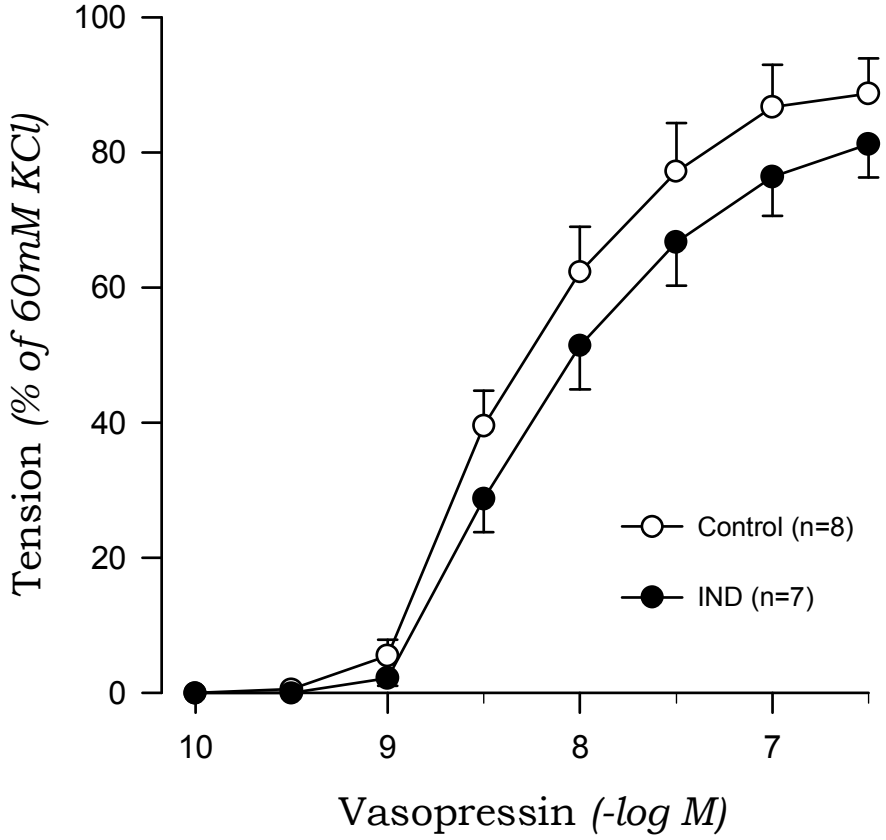

Fig. 10. Effects of indapamide (IND) on contractile responses to vasopressin in aortic rings from sham-operated rats.

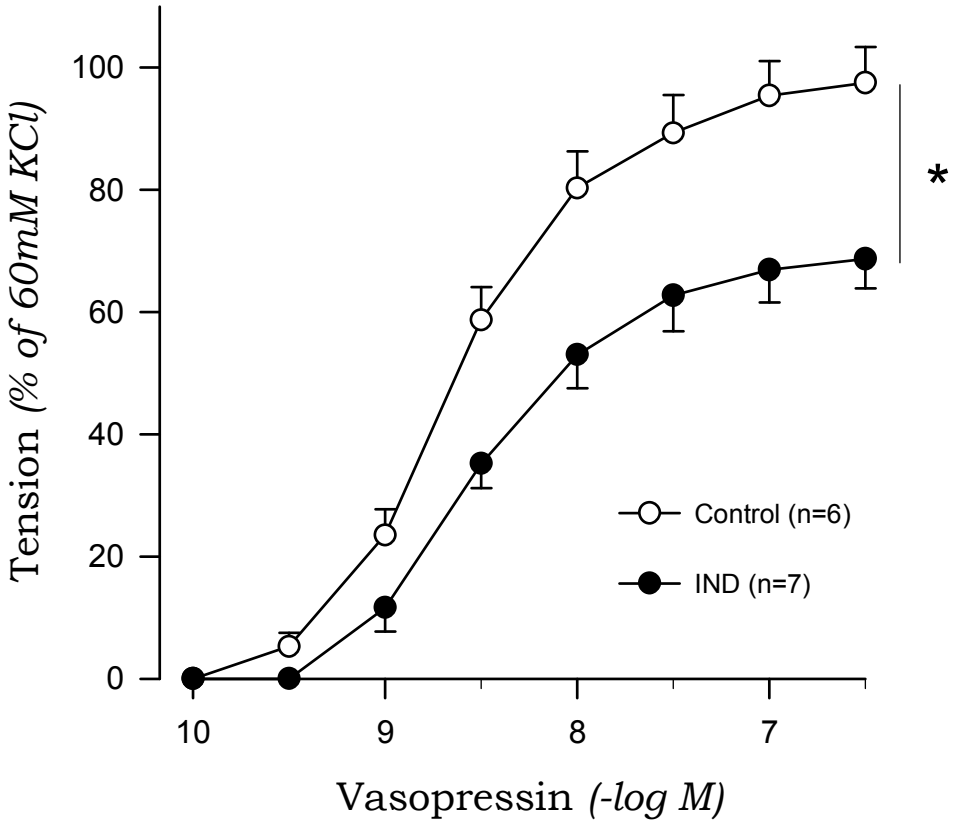

Fig. 11. Effects of indapamide (IND) on contractile responses to vasopressin in aortic rings from 2K1C hypertensive rats. *P<0.05, compared with the control value.

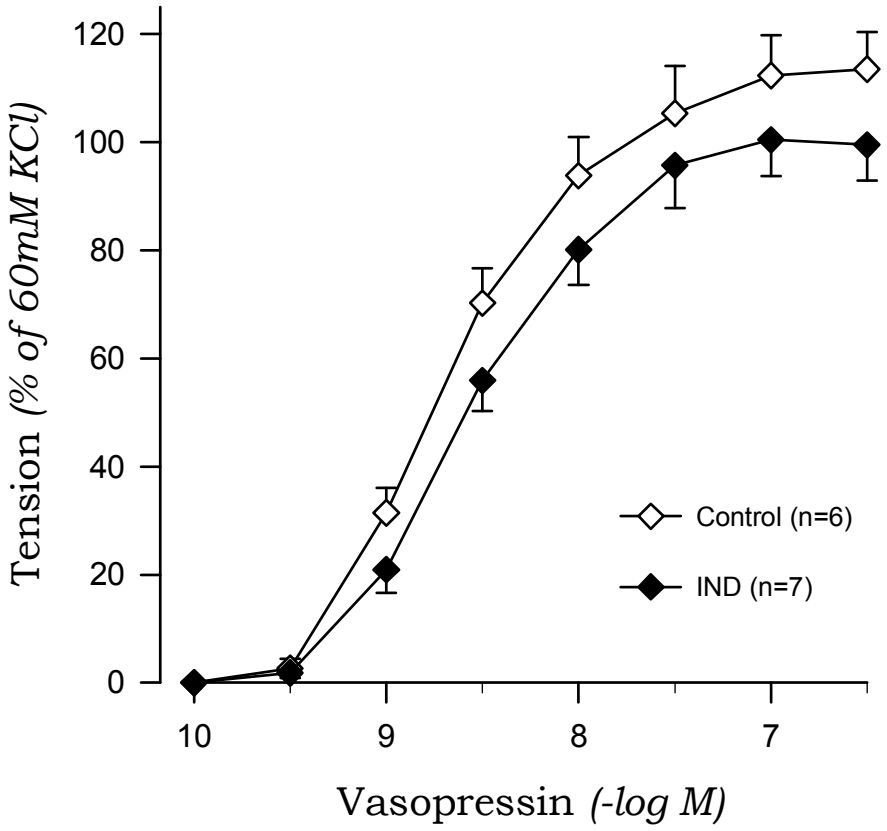

Fig. 12. Effects of indapamide (IND) on contractile responses to vasopressin in aortic rings without endothelium from 2K1C hypertensive rats.

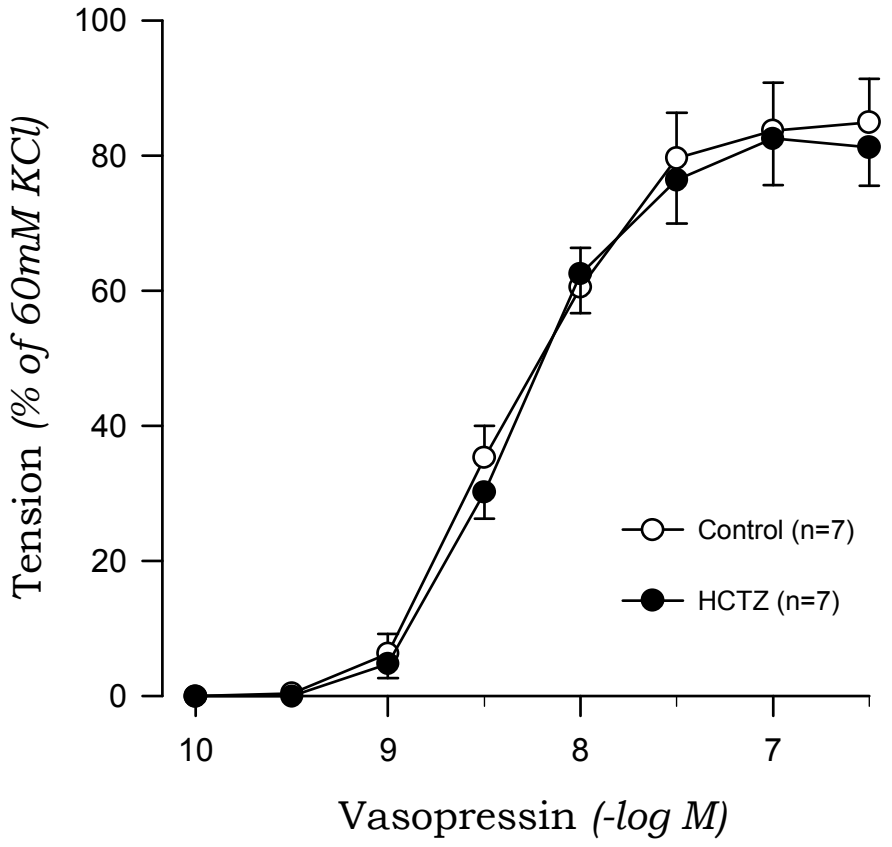

Fig. 13. Effects of hydrochlorothiazide (HCTZ) on contractile responses to vasopressin in aortic rings from sham-operated rats.

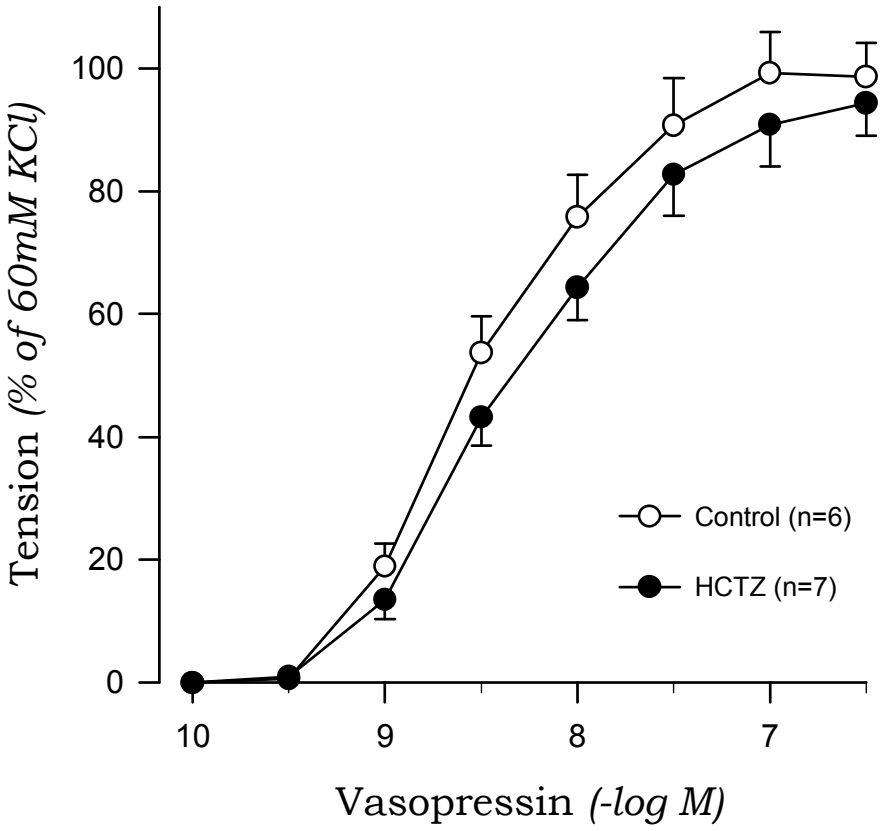

Fig. 14. Effects of hydrochlorothiazide (HCTZ) on contractile responses to vasopressin in aortic rings from 2K1C hypertensive rats.

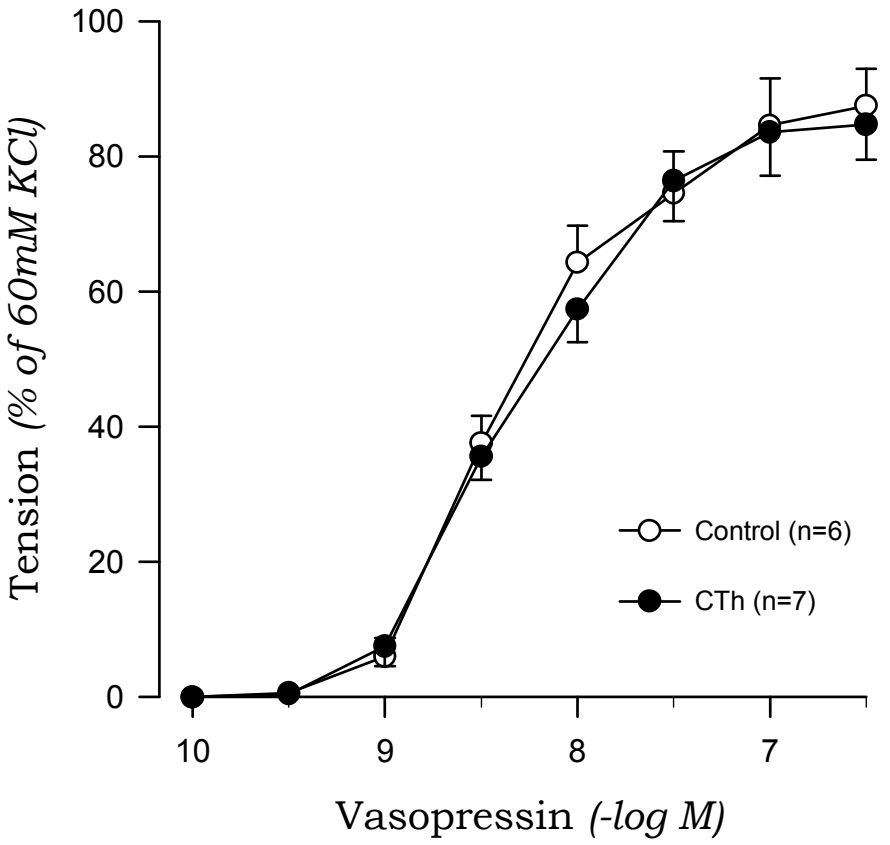

Fig. 15. Effects of chlorthalidone (CTh) on contractile responses to vasopressin in aortic rings from sham-operated rats.

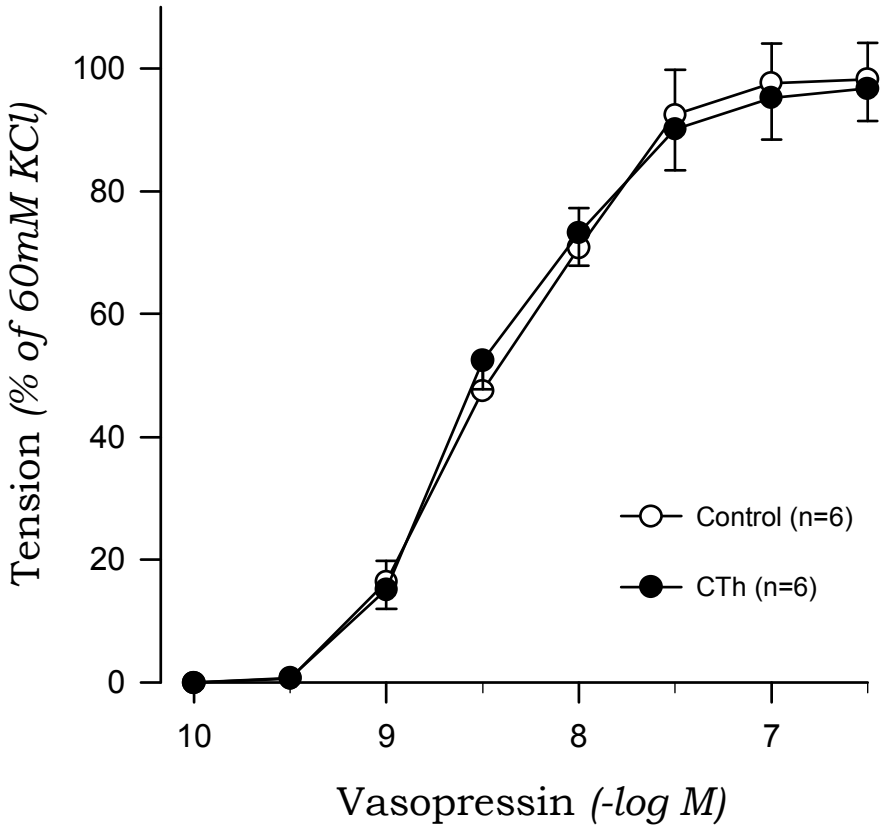

Fig. 16. Effects of chlorthalidone (CTh) on contractile responses to vasopressin in aortic rings from 2K1C hypertensive rats.

Ⅳ. DISCUSSION

Previous studies have observed that vascular responsiveness to contractile agonist is enhanced in various models of hypertension[16,17]. In the present study, similar to our previous findings[13,14], the pD_2 values for norepinephrine and vasopressin were significantly higher in aortic rings from 2K1C hypertensive rats as compared to those from sham-clipped control rats. Enhanced contractile responses in hypertension may be attributed to an augmented phosphoinositide hydrolysis, a greater release of intracellular Ca^{2+} from a cellular pool, an increased activation of protein kinase C, or alterations in number of affinity of inositol trisphosphate receptors on the endoplasmic reticulum of vascular smooth muscle[18]. Many of these processes may also be responsible for the increased sensitivity of vascular response to agonist in 2K1C hypertension.

Indapamide, a thiazide-related diuretics, is an orally active sulfonamide agent with antihypertensive properties which has been shown to exert effective control of blood pressure in animals[9,11] and human[19]. It reduces arterial blood pressure by exerting a diuretic effect and by inducing relaxation of vascular smooth muscle[20]. In the present study, we found that indapamide 10^{-4} M inhibited norepinephrine and vasopressin-induced contractions. The inhibitory effect of indapamide was observed only in endothelium-intact aortic rings

from 2K1C rats, but is ineffective on rings from which endothelium had been removed. These results suggest that effects of indapamide are mediated by the endothelium. Similar results were reported by Colas et al.[11] who have observed that the contractile response induced by norepinephrine or vasopressin are inhibited by methyclothiazide, a thiazide diuretic, in aortic rings from spontaneously hypertensive rats. They have also found that the inhibitory effect of methyclothiazide is attenuated by either mechanical removal of the endothelium or pretreatment with Nω-nitro-L-arginine[10], a nitric oxide synthase inhibitor, and speculated that methyclothiazide may exert its action by an endothelium-dependent mechanism.

The endothelium regulates vascular tone through the release of several factors that modulate the contractile activity of the underlying smooth muscle[21]. Therefore, the endothelium is a favorite early target of cardiovascular risk factors and cardiovascular diseases like hypertension[12]. It has been proposed that various forms of experimental hypertension[22] including 2K1C hypertension[13] are characterized by a dysfunctional endothelium. A large portion of the studies on endothelial dysfunction have concentrated on the mechanisms of reduced endothelium-dependent relaxations mainly results from an increase in nitric oxide degradation, in hypertension[12]. In the present study, however, the contractile response induced by norepinephrine and vasopressin were attenuated by indapamide only in intact aortic rings from 2K1C rats. Our results imply

that the blood pressure lowering activity of indapamide is supported by a reduction of the vascular response to endogenous vasoconstricting stimuli such as norepinephrine or vasopressin, via an endothelium-dependent mechanism in hypertensive state. Taken together, as has been demonstrated previously[10,11], it is speculated that vasodilator factor released from the endothelium is promoted after indapamide treatment, possibly may be a compensatory mechanism developed to offset the diminished relaxant responsiveness of smooth muscle cells in 2K1C renal hypertension. In support to this notion, it has been found that indapamide and its hydroxy-metabolite have a free-radical scavenging activity[23] and protects the degradation of nitric oxide[24], endothelium-derived relaxing factor.

In the present study, the inhibitory effect of indapamide was observed only with the highest concentration of the drug tested, much higher than human blood concentration (80 nM) obtained during chronic utilization[11]. One possible explanation for this finding is that indapamide is very poorly soluble in aqueous solution, which may lead to overestimation of the amount of the drug actually in solution under the experimental conditions tested in this study. In addition, in vivo, indapamide acts as a prodrug, as one of the major metabolites of indapamide [the 5-OH derivative], contributes to the antihypertensive and vasodilator action of indapamide and possesses, in vitro, a more intense antioxidant activity than the parent compound[23]. It is unlikely that such active metabolites would be formed in our

experimental conditions with isolated blood vessels.

Possible limitations of this study include the use of the rat aorta as an experimental model, which contributes little to vascular resistance. However, although it is generally accepted that the primary abnormality in essential hypertension is an increase in peripheral resistance, it has been proposed that an increase in stiffness of large blood vessels is of equal importance in hypertension[25]. In this proposal, loss of flexibility in capacitance vessels is the predominant abnormality, and it has also been found that the endothelial dysfunction is detected functionally both in resistance and conduit arteries in various model of experimental hypertension[12]. On the basis of this information, as has been shown previously[10,11], we think that capacitance vessels have an under-recognized role in hypertension and that the rat aortic ring preparation is a reasonable model for this phenomenon. In addition, the previous study have also mentioned that rat aortic ring preparation is the most sensitive preparation with respect to the vasorelaxant action of the diuretics tested[26].

In contrast to indapamide, incubation with hydrochlorothiazide or chlorthalidone did not change the sensitivity to norepinephrine and vasopressin in not only sham-clipped but 2K1C hypertensive rat aortic rings. Similar results were also observed previously in aortic rings from spontaneously hypertensive rats[11]. In accordance with these observations, it has been found that pressor responses to intravenous norepinephrine are reduced

by pretreatment with indapamide in experimental hypertensive animals, while cardiovascular activity is unaffected by hydrochlorothiazide treatment[27]. Although it has been shown that the vasorelaxant action of indapamide is more potent than hydrochlorothiazide or chlorthalidone[9,26], hydrochlorothiazide is an orally active diuretic agent which is used in mild to moderate hypertension[2]. Moreover, previous studies have reported that hydrochlorothiazide causes relaxation of isolated human and guinea-pig small mesenteric resistance vessels[8]. However, this same group was unable to demonstrate any relaxant activity of hydrochlorothiazide on isolated rat mesenteric vessels[8]. Thus, it is difficult to interpret a difference of the effects of hydrochlorothiazide or chlorthalidone as compared with indapamide in our study. The possible discrepancy may be ascribed to diuretics have different actions on different species and tissues[26].

In summary, our results provide evidence that antihypertensive effect of indapamide, in addition to its diuretic effect, is mediated by reduction of the vascular response to the action of endogenous vasoconstricting stimuli such as norepinephrine and vasopressin via an endothelium-dependent mechanism in 2K1C hypertensive rats. An enhanced vasorelaxant effect of indapamide in intact aortic rings from 2K1C rats imply that vasodilator factor was promoted or vasoconstrictor factor release from the endothelium was diminished after indapamide treatment. This may possibly be a compensatory mechanism

developed to offset the diminished relaxant or enhanced contractile responsiveness of smooth muscle cells in 2K1C hypertension. Thus, such combinations may be of a value in the treatment of hypertension.

V. SUMMARY

Thiazide diuretics exert their hypotensive efficacy through a combined vasodilator and diuretic effect. The present study was conducted to assess the inhibitory effect of thiazide diuretic, hydrochlorothiazide, and the thiazide-like diuretics, indapamide and chlorthalidone on contractile responses to norepinephrine and arginine vasopressin in aortic rings from 2K1C renal hypertensive and sham-clipped normotensive rats. 2K1C hypertension was made by clipping the left renal artery and age-matched control rats received a sham treatment. Changes in the tension of aortic ring preparations were measured isometrically. Indapamide inhibits the contractile responses to norepinephrine and vasopressin in aortic rings from 2K1C rats, while it did not modify in control rats. The inhibitory effect of indapamide was abolished by endothelium removal. Hydrochlorothiazide or chlorthalidone did not affect the vasoconstriction induced by norepinephrine and vasopressin either in sham or in 2K1C hypertensive rats. These results suggest that indapamide inhibits the contractile responses to norepinephrine and vasopressin via an endothelium-dependent mechanism in 2K1C renal hypertension.

REFERENCES

1) Duarte JD, Cooper-DeHoff RM: Mechanisms for blood pressure lowering and metabolic effects of thiazide and thiazide-like diuretics. *Expert Rev Cardiovasc Ther* 8:793-802, 2010

2) Nash DT: Rationale for combination therapy in hypertension management: focus on angiotensin receptor blockers and thiazide diuretics. *South Med J* 100:386-392, 2007

3) Freis ED: How diuretics lower blood pressure. *Am Heart J* 106:185-187, 1983

4) Reungjui S, Roncal CA, Mu W, Srinivas TR, Sirivongs D, Johnson RJ, Nakagawa T: Thiazide diuretics exacerbate fructose-induced metabolic syndrome. *J Am Soc Nephrol* 18:2724-2731, 2007

5) Velazquez H, Wright FS, Good DW: Luminal influences on potassium secretion: chloride replacement with sulphate. *Am J Physiol* 242:F46-F55, 1982

6) Schiffl H, Weidmann P, Meier A, Ziegler WH: Relationship between plasma catecholamines and urinary catecholamine excretion rates in normal subjects and certain diseased states. *Klin Wochenschr* 59:837-844, 1981

7) Van Brummelen P, Man in't Veld AJ, Schalekamp MA: Hemodynamic changes during long term thiazide treatment of essential hypertension in responders and nonresponders.

Clin Pharmacol Ther 27: 328-336, 1980

8) Calder JA, Schachter M, Sever PS: Direct vascular actions of hydrochlorothiazide and indapamide in isolated small vessels. *Eur J Pharmacol* 220:19-26, 1992

9) Abrahams Z, Pang MY, Lam EK, Wright JM: What is the plasma cofactor required by diuretics for direct vascular relaxant effect in vitro? *J Hypertens* 16:801-809, 1998

10) Colas B, Slama M, Collin T, Safar M, Andrejak M: Mechanisms of methyclothiazide-induced inhibition of contractile responses in rat aorta. *Eur J Pharmacol* 408:63-67, 2000

11) Colas B, Slama M, Masson H, Colas JL, Collin T, Amould ML, Hary L, Safar M, Andrejak M: Direct vascular actions of methyclothiazide and indapamide in aorta of spontaneously hypertensive rats. *Fundam Clin Pharmacol* 14:363-368, 2000

12) Thuillez C, Richard V: Targeting endothelial dysfunction in hypertensive subjects. *J Hum Hypertens* 19(*Suppl* 1):21-25, 2005

13) Jun JY, Yeum CH, Moon SH, Cho CH, Jun KB, Chung JH, Yoon PJ: Altered endothelial modulation of vasoconstriction in chronic two-kidney, one clip hypertensive rats. *Kor J Nephrol* 20:381-392, 2001

14) Choi S, Jun KB, Jun JY, Yoon PJ, Kim JS, Kim MY, Mun HS, Yeum CH: Effect of Aminoguanidine on norepinephrine-Induced vascular contraction in renovascular

hypertensive rats. *Kor J Nephrol* 23:703-713, 2004
15) Sigmon DH, Beierwaltes WH: Influence of nitric oxide in the chronic phase of two-kidney one clip renovascular hypertension. Hypertension 31:649-656, 1998
16) Webb RC, Bohr DF: The membrane of the vascular smooth muscle cell in experimental hypertension and its response to serotonin. in: *Smooth Muscle Contractions*. N Stephens, New York p485-508, 1983
17) Bohr DF, Dominiczak AF, Webb RC: Pathophysiology of the vasculature in hypertension. *Hypertension* 18(*Suppl* Ⅲ):69-75, 1991
18) Turla MB, Webb RC: Augmented phosphoinositide metabolism in aortas from genetically hypertensive rats. *Am J Physiol* 258:H173-H178, 1990
19) Patel A; ADVANCE Collaborative Group, MacMahon S, Chalmers J, Neal B, Woodward M, Billot L, Harrap S, Poulter N, Marre M, Cooper M, Glasziou P, Grobbee DE, Hamet P, Heller S, Liu LS, Mancia G, Mogensen CE, Pan CY, Rodgers A, Williams B: Effects of a fixed combination of perindopril and indapamide on macrovascular and microvascular outcomes in patients with type 2 diabetes mellitus (the ADVANCE trial): a randomised controlled trial. *Lancet* 370:829-840, 2007
20) Chaffman M, Heel RC, Brogden RN, Speight TM, Avery GS: Indapamide: A review of its pharmacodynamic properties and therapeutic efficacy in hypertension. *Drugs*

28:189-235, 1984
21) Vane JR, Anggard EE, Botting RM: Regulatory functions of the vascular endothelium. *N Engl J Med* 323:27-36, 1990
22) Luscher TF, Raij L, Vanhoutte PM: Endothelium-dependent vascular responses in normotensive and hypertensive Dahl rats. *Hypertension* 9:157-163, 1987
23) Tamura A, Sato T, Fujii T: Antioxidant activity of indapamide and its metabolite. *Chem Pharm Bull* 38:255-257, 1990
24) Rubanyi GM, Vanhoutte PM: Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor. *Am J Physiol* 250:H822-H827, 1986
25) Franklin SS, Weber MA: Measuring hypertensive cardiovascular risk: the vascular overload concept. *Am Heart J* 128:793-803, 1994
26) Finch L, Hicks PE, Moore RA: Changes in vascular reactivity in experimental hypertensive animals following treatment with indapamide. *J Pharm Pharmacol* 29:739-743, 1977
27) Abrahams Z, Tan LL, Pang MY, Abrahams B, Tan MM, Wright JM: Demonstration of an in vitro direct vascular relaxant effect of diuretics in the presence of plasma. *J Hypertens* 14:381-388, 1996