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Effects of Norepinephrine and Neuropeptide Y on the Contractility of Small Mesenteric Artery from 2K1C and DOCA-Salt Hypertensive Rats

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The present study was conducted to investigate the possible role of the sympathetic nervous system in two-kidney, one clip (2K1C) and deoxycorticosterone acetate (DOCA)-salt hypertension. 2K1C and DOCA-salt hypertension were made in Sprague-Dawley rats. Four weeks after induction of hypertension, systolic blood pressure measured in conscious state was significantly higher in 2K1C (216±18 mmHg) and DOCA-salt (205±29 mmHg) groups than that in control (128±4 mmHg). The third branches (<300 μm in outer diameter) of the mesenteric artery were isolated and cut into ring segments of 2~3 mm in length. Each ring segment was mounted in tissue bath and connected to a force displacement transducer for measurement of isometric tension. The arterial rings were contracted by application of norepinephrine (NE) in a dose-dependent manner. The amplitude of the NE-induced contraction of the vessels was significantly larger in hypertension than in control. The NE-induced contraction was significantly enhanced by neuropeptide Y (NPY) in hypertension. Reciprocally, NPY-elicited vasocontraction was increased by NE in hypertension. These results suggest that the sympathetic nervous system contributes to the development of 2K1C and DOCA-salt hypertension.

Key Words: Small mesenteric artery, Two-kidney, one clip hypertension, Deoxycorticosterone acetate-salt hypertension, Norepinephrine, Neuropeptide Y

INTRODUCTION

An enhanced activity of the renin-angiotensin system and volume expansion have been implicated in the development of two-kidney, one clip (2K1C) and deoxycorticosterone acetate (DOCA)-salt hypertension, respectively. Thus the sympathetic nervous system is unlikely involved in pathophysiological cause of these types of hypertension. However, the possible role of peripheral adrenergic nerve factors in the vascular reactivity associated with 2K1C hypertension was observed (Katholi et al, 1982). Furthermore the development of 2K1C hypertension was effectively arrested by splanchnicotomy and/or injection of ganglionic blocker, chlorisondamine (Nakada et al, 1996). In addition to its possible role in 2K1C, the vasoconstrictor responses to exogenous NE were significantly increased in DOCA-salt hypertension (Masuyama et al, 1986). Plasma norepinephrine (NE) and neuropeptide Y (NPY)-like levels also increased significantly in parallel with blood pressure during DOCA-salt treatment, indicating blood pressure was significantly correlated with plasma norepinephrine and NPY-like levels in DOCA-salt hypertensive rats (Moreau et al, 1992). Although these results suggest that the sympathetic nervous system is involved in 2K1C and DOCA-salt hypertensions, its precise role in the hypertension models has not been fully understood. NE and NPY are known to be co-localized in sympathetic neurons (Lundberg et al, 1983) and co-released from the nerve terminals (Hass et al, 1989). Although

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increased vascular reactivity to NE was observed in 2K1C hypertension (Fortes et al, 1990) and DOCA-salt hypertensive hypertension (Masuyama et al, 1986), little has been known about the role of NPY, especially its interaction with NE. The NE effects on the NPY-induced vascular response, which means the adrenergic modulation of the NPY vascular action, has been recently examined in normal rats (Cortes et al, 1999), not in 2K1C and DOCA-salt hypertension. The purpose of this study was to explore the role of sympathetic neurotransmitters in regulating the vascular tone in 2K1C and DOCA-salt hypertension. The effects of NE and NPY on the vascular contractility were investigated in the small mesenteric artery isolated from both hypertensive models.

METHODS

Male Sprague-Dawley rats weighing 150~180 g were used. 2K1C hypertension was induced by clipping the left renal artery with a 0.2 mm silver clip under ketamine (50 mg/kg, IP) anesthesia. To develop DOCA-salt hypertension, rats were subcutaneously implanted DOCA strip one week after the unilateral nephrectomy, and supplied 1.0% saline as drinking water thereafter. Sham-operated rats without clipping or implanting the DOCA strip served as control. They were used 4 weeks later.

On the day of experiment, systolic blood pressure was indirectly measured by tail-cuff method in a conscious state. It was significantly higher in 2K1C (216 ± 18 mmHg, n=15, $p<0.01$) and DOCA-salt rats (205 ± 29 mmHg, n=12, $p<0.01$) than in the control (126 ± 4 mmHg, n=14).

Rats were then sacrificed using a guillotine. The mesenteric arteries were removed and placed in cold physiological salt solution (PSS) containing (mM) NaCl 130, KCl 4.7, KH_2PO_4 1.18, $MgSO_4$ 1.17, NaHCO3 14.9, $CaCl_2$ 1.6, dextrose 5.5, and EDTA 0.03. The pH was adjusted to 7.3 with NaOH. After dissection of adhering tissues under a microscope, the third branches (<300 μm in outer diameter) of the arteries were gently removed and immersed in cold oxygenated PSS solution.

The vessels were cut into cylindrical segments 2~3 mm in length. Each cylindrical segment was carefully mounted on two triangle-shaped metal rings. One of which was connected to a force displacement transducer (model FT03C, Grass, U.S.A.) for continuous recording of isomeric tension, the other to a hooked bar in tissue bath. The position of the holder could be changed by means of movable unit allowing fine adjustments of vascular resting tension by varying the distance between the force transducer and the hook on the bar. The mounted vessel segment was immersed in temperature controlled 5 ml tissue bath containing PSS solution aerated with 5% CO_2 −95% O_2 at 37°C. A tension of 0.5 or 0.75 g was applied to each segment and it was stabilized at this level of tension for 1.5 hour.

Isometric tension was recorded with a force dis-

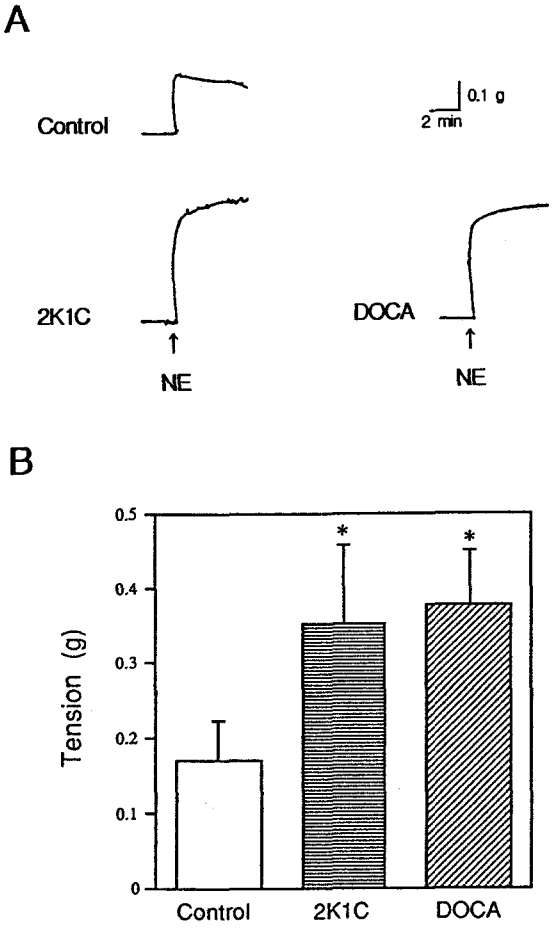

Fig. 1. Vascular contraction induced by norepinephrine in hypertension and control. A. Contraction of the small mesenteric artery was evoked by application of norepinephrine (NE, 10 μM) at the times indicated by arrows in normotension (Control) and hypertension (2K1C and DOCA). B. Average magnitude of the vascular contraction generated by NE (10 μM) in control (n=15), 2K1C (n=30) and DOCA (n=21). Data were the mean±SD. *$p<0.05$; compared with control.

placement transducer (FT03C) connected to an amplifier (model 7P1G, Grass) and displayed on a Grass ink-writing polygraph (model 79E). The magnitude of arterial tension induced by either NE or NPY in this study could not be normalized with each arterial weight since the small mesenteric artery was too light to measure (<0.1 mg). All chemicals including NE and NPY were obtained from Sigma Chemical Company (St. Louis, MO, U.S.A.).

Data were presented as mean values±SD. Statistical significance of the results was determined using unpaired Student's *t* test for the effects of NE on the vascular contraction and paired Student's *t* test for the effects of NE or NPY on the NPY- and NE-induced vascular contraction, respectively. A value of $p<0.05$ was considered as significant.

RESULTS

The exogenously applied NE elicited fast and long-lasting contraction of the arterial rings compared with that evoked by NPY, as is demonstrated in recording taken from a typical experiment showing the arterial response to NE (10 μM, Fig. 1A). The amplitude of the NE-induced contraction was significantly larger in 2K1C and DOCA-salt hypertension than in control (Fig. 1B). The arterial segments from three groups responded to NE application in a dose-dependent manner (Fig. 2). The segments contracted when continuously exposed to increasing concentrations from 0.1 to 10 μM of NE.

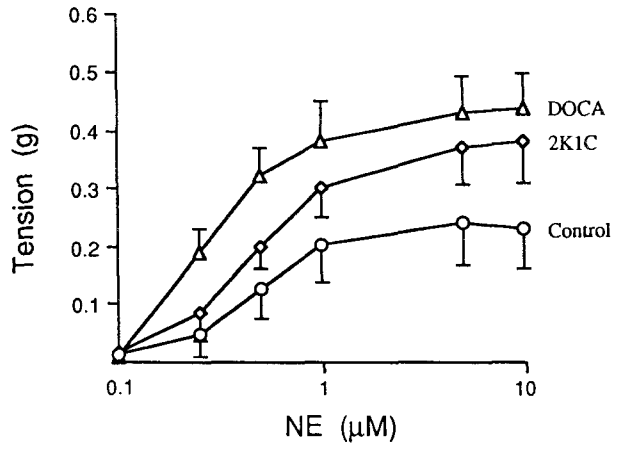

Fig. 2. Dose-response relationships for the norepinephrine-induced contraction of the isolated mesenteric arteries in hypertension (2K1C, DOCA) and control. Vascular contraction was induced by cumulative applications of norepinephrine of 0.1, 0.25, 0.5, 1, 5, and 10 μM. Each point is the mean±SD of 8 to 14 experiments.

The effects of NPY on the NE-induced vascular contraction was investigated. The magnitude of the arterial contraction generated by a low concentration

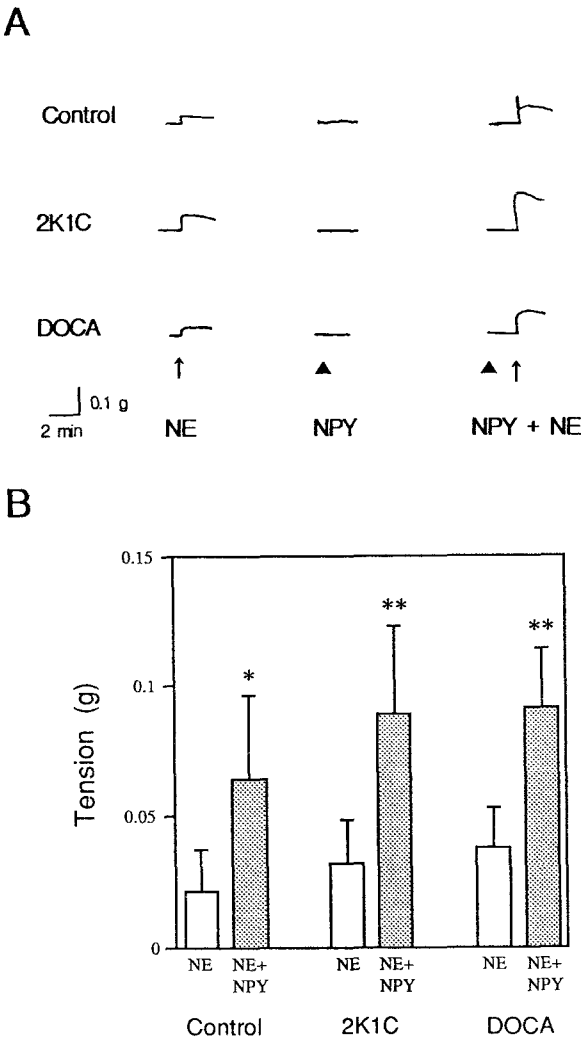

Fig. 3. Effects of neuropeptide Y on the vascular contraction evoked by a low concentration of norepinephrine. A. Vascular contraction induced by norepinephrine (NE; 250 nM), neuropeptide Y (NPY; 50 nM) and NE (250 nM) in the presence of NPY (NPY+NE; 50 nM) in control and hypertension (2K1C, DOCA). Arrows and closed triangles represent the application time of NE and NPY, respectively. B. Average magnitude of the NE-induced vascular response by NPY in three groups. Mean values±SD are shown, n=5~12 for each group. *$p<0.05$, **$p<0.001$; compared with that in the absence of NPY using paired *t* test.

(250 nM) of NE was significantly enhanced in the presence of NPY with similar increasing rates in control, 2K1C, and DOCA hypertensive groups (Fig. 3). The vascular contraction induced by a high concentration (500 nM) of NE was also significantly increased by preapplication of NPY in two hypertension groups, whereas it was not significantly affected by NPY in control group (Fig. 4). In all groups, the rate of the NPY-induced increase in the vascular contraction evoked by NE was inversely related to its contraction magnitude.

In addition to the effects of NPY on the NE-induced contraction, the effects of NE on the vascular response to NPY was also explored. The vascular contraction with minimal response generated by a low concentration (50 nM) of NPY was remarkably increased

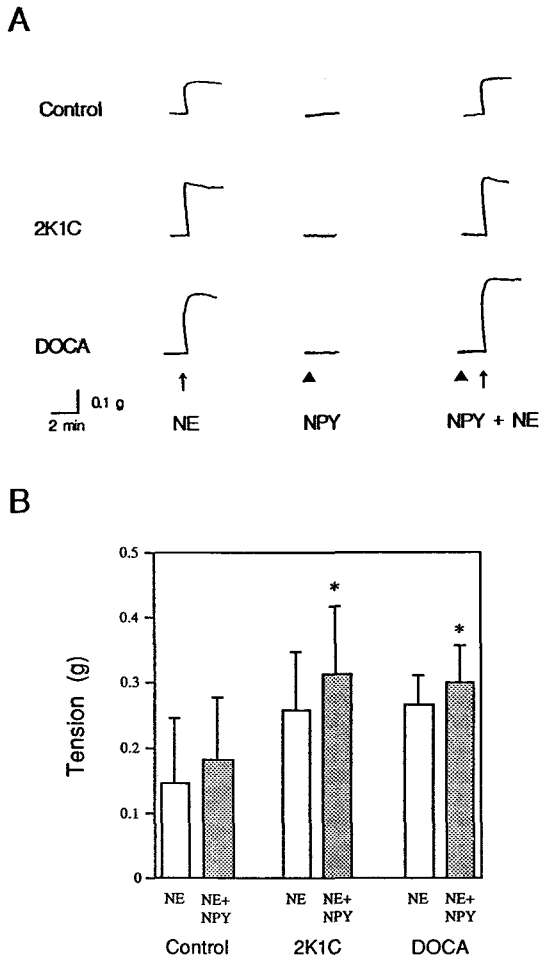

Fig. 4. Neuropeptide Y effects on the vascular response to a high concentration of norepinephrine. A. Vascular contraction induced by norepinephrine (NE; 500 nM), neuropeptide Y (NPY; 50 nM) and NE (500 nM) in the presence of NPY (NPY+NE; 50 nM) in control and hypertension (2K1C, DOCA). Arrows and closed triangles represent the application time of NE and NPY, respectively. B. Average magnitude of the NE-induced vascular response by NPY in three groups. Mean values ±SD are shown, n=5~10 for each group. *$p<0.01$; compared with that in the absence of NPY using paired *t*-test.

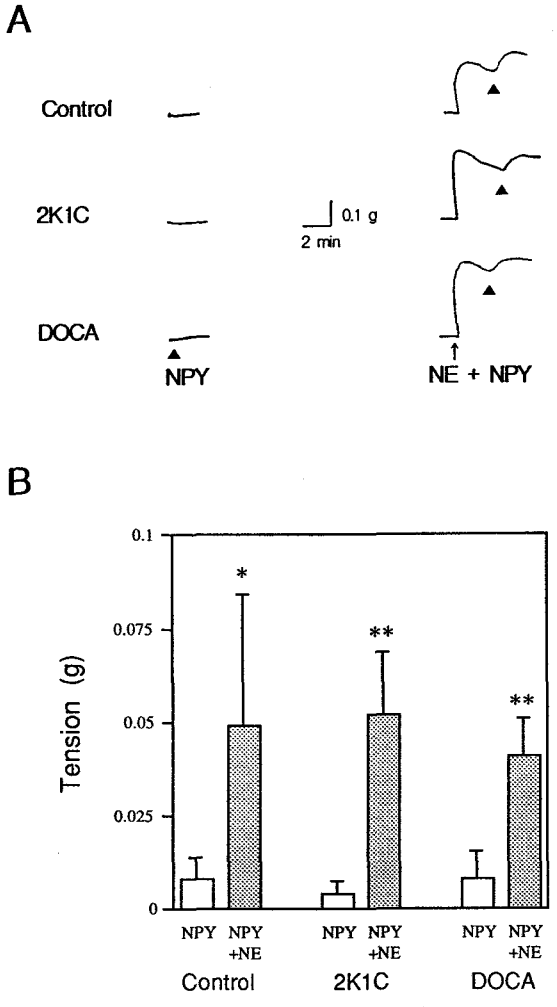

Fig. 5. Effects of norepinephrine on the neuropeptide Y-induced vascular contraction. A. Vascular response induced by neuropeptide Y (50 nM) in the absence of 500 nM norepinephrine (NPY) and in its presence (NE+NPY) in control and hypertension (2K1C, DOCA). Neuropeptide Y was applied at the times indicated by closed triangles after application of norepinephrine. An arrow indicates the time of NE application. B. Average amplitude of the NPY-induced vascular response in the presence of NE in three groups. Mean values±SD are shown, n=7~12 for each group. *$p<0.05$, **$p<0.001$; compared with that in the absence of NE using paired *t* test.

in the presence of NE in all three groups (Fig. 5).

DISCUSSION

The present study demonstrates that sensitivity of the small artery to NE in 2K1C and DOCA hypertension is increased and that the NE-induced contraction is potentiated by NPY and vise versa. Our observation was in agreement with previous investigations showing the increased vascular response to NE. For example, enhanced reactivity to NE was observed in aortae and mesenteric microvessels isolated from 2K1C hypertensive rats (Fortes et al, 1990; Deng & Schiffrin, 1991). The vasoconstrictor responses to exogenous NE were significantly increased in DOCA hypertensive rat models compared with their age-matched normotensive controls (Masuyama et al, 1986). Although similar vascular sensitivity to NE was also observed in DOCA-salt hypertension and control (Hermsmeyer et al, 1982), the different size of artery was probably responsible for the negative result. Moreover, basal NE levels in DOCA hypertension were about twice greater than those found in normotensive control (Bouvier & de Champlain, 1986). The elevated plasma NE levels in DOCA-salt animals were significantly correlated with their mean arterial pressures (Bouvier & de Champlain, 1985). The pressor responses to electrical nerve stimulation were also greater in DOCA-salt hypertension than in control (Masuyama et al, 1986).

Potentiation of the NE effect on the small artery by NPY with a low concentration in renal hypertensive groups was observed. NPY in the control group enhanced the vascular contraction that was induced by a low concentration of NE only, whereas NPY in hypertension groups potentiated the vascular response that was evoked by either a low or a high concentration of NE. These findings are in line with the evidence that a low concentration of NPY enhanced a submaximal response to exogenous NE 2.8 times in the small mesenteric artery of rats, but it did not cause a contraction in the absence of NE (Sjoblom-Widfeldt et al, 1990). NPY-induced potentiation of NE effects in the mesenteric microvessels of normal rats was also observed without statistical significance (Chen et al, 1996). Different concentration of NPY used in each study could be explained for the discrepancy. The speculation can be supported by the results from different types of vessels (Pernow, 1988) and experiments (Sjoblom-Widfeldt et al, 1990). Enhancement of the NE-induced contraction in rat femoral artery was evoked by a low concentration of NPY (Pernow, 1988). Single twitch of small mesenteric artery evoked by nerve stimulation was increased by NPY in low concentration (Sjoblom-Widfeldt et al, 1990). Recently, it has been demonstrated that NPY is released from the perimesenteric arterial sympathetic nerves and that its action was occurred via the activation of NPY-Y1 receptors (Bergdahl et al, 1996; Malmstrom, 1997). NPY is responsible for the potentiation of NE effect on perfusion pressure in the isolated rat mesenteric bed (Donoso, 1997). Moreover, the voltage-dependent Ca^{2+} channel blocker nitrendipine (300 nM) did not affect the potentiation response to NPY, suggesting that Ca^{2+} entry through the Ca^{2+} channels is not associated with the NPY-induced potentiation (Chen et al, 1996).

In addition to the potentiation effects of NPY, a direct role of NPY in vasoconstriction evoked by nerve stimulation has been demonstrated. Sympathetic nerve stimulation produced a frequency-dependent increase in perfusion pressure and concomitant overflow of NPY immunoreactivity in the perfusate (Han et al, 1998). Increased NPY release from sympathetic nerve terminal, resulting in an increased plasma NPY level, was observed in primary hypertension and DOCA-salt hypertension. In the superior mesenteric artery, NPY-immunoreactivity content was significantly higher in 4-week-old but lower in 16-week-old SHR than in WKY rats, suggesting greater NPY stores and release during the development of hypertension (Zukowska et al, 1993). In DOCA-salt hypertension, increased plasma NPY-like immunoreactivity levels originated primarily from the sympathetic nerves, because those levels were correlated exclusively with circulating NE levels and they were associated with a reduction in NPY-like immunoreactivity content of the heart and mesenteric artery (Moreau et al, 1992). The plasma concentrations of NPY correlated with the degree of fluid overload and the mean arterial blood pressure in hemodialysis patients having large amounts of fluid overload (Odar-Cederlof et al, 1998).

Reciprocally, the NPY-evoked response was remarkably potentiated by NE in the control, 2K1C and DOCA-salt hypertension groups. NPY of a low concentration was unable to contract the mesenteric artery, whereas it constricted the precontracted vessel with NE (Cortes et al, 1999). Taken together, the

sympathetic nervous system may contribute to 2K1C and DOCA-salt hypertension by various ways including different vascular sensitivity to sympathetic neurotransmitters, different amount of neurotransmitters, and different interactions of neurotransmitters.

In summary, the augmented sensitivity of the small artery to NE and the potentiation of the NE effects by NPY and vice versa were observed in 2K1C and DOCA-salt hypertension. However, our previous studies demonstrated that the vascular smooth muscle in 2K1C has different properties related to intracellular calcium regulation compared with that in DOCA-salt hypertension (Kim et al, 1997; Nam et al, 1999). Thus, further studies including the effects of NE, NPY, adenosine 5'-triphosphate alone and in combination on microvessel contraction as well as cellular mechanisms will be needed to elucidate the precise role of the sympathetic activity in each type of hypertension.

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