INTRODUCTION

Thiazide diuretics are efficacious in lowering elevated blood pressure and effective in reducing morbidity and mortality in patients with mild to severe hypertension. 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. Hemodynamically, the diuretic effect of thiazides is known to decrease blood pressure primarily by reducing extracellular fluid volume and cardiac output. 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.

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. Several studies have demonstrated a direct vascular action of the thiazide diuretics, hydrochlorothiazide or methyclothiazide, and the thiazide-like diuretics, indapamide and chlorthalidone.

Previous studies have shown that methyclothiazide
inhibits the contractile response induced by norepinephrine in spontaneously hypertensive rat aorta, only when the endothelium is present\textsuperscript{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\textsuperscript{12}. We have also observed that the role of endothelium is impaired in two–kidney, one clip (2K1C) renal hypertension\textsuperscript{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\textsuperscript{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 chlorothalidone 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.

**MATERIALS AND 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 contra-lateral 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 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\textsuperscript{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\textsubscript{3} 25, MgCl\textsubscript{2} 1.2, KH\textsubscript{2}PO\textsubscript{4} 1.2, CaCl\textsubscript{2} 2.5 and glucose 11.1. The vessels were cleaned of 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°C, and bubbled with a mixture of 95% O\textsubscript{2} and 5% CO\textsubscript{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 FTO\textsubscript{3}) for measurement of isometric tension. 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...