Vascular reactivity in chronic Goldblatt two kidney-one clip hypertensive rats

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Summary. We studied the possible contribution of increased vascular reactivity in the chronic phase of Goldblatt two kidney-one clip hypertension. Vascular reactivity was evaluated in aortic strips from hypertensive rats (16 weeks after inducing hypertension) and age-matched control rats. The findings were: a) increased sensitivity to vasopressin in the aortic tissue of hypertensive rats, b) a similar response to angiotensin II, noradrenaline and KCl in hypertensive and control rats, and c) reduced maximal response to angiotensin II compared with other vasoconstrictors in both groups of rats. These results suggest a possible role for vasopressin in the chronic phase of this model of hypertension.
Key words. Goldblatt two kidney-one clip hypertension; vascular reactivity; noradrenaline; vasopresin; angiotensin.

Goldblatt two kidney-one clip (G 2k–1c) hypertension is initiated by hyperactivity of the renin-angiotensin system (renin-dependent model) secondary to renal ischemia. Hemodynamically, an increased peripheral resistance of the vascular system is a major characteristic of the initial phase of this model of hypertension [2]. Several mechanisms may raise vascular resistance in hypertension; structural alterations [3], rarefaction [4], increased neurohumoral factors [5, 6] and increased reactivity to vasoconstrictors [7, 8]. Few reports have appeared in the literature on vascular reactivity in G 2k–1c hypertensive rats, and the reports published to date are conflicting. Whereas Bandick and Sparks [7] have shown an increased vasoconstrictor reactivity, others have shown either no change [9] or a decreased response [10]. The purpose of the present study was to determine whether aortic tissue of G 2k–1c hypertensive rats in the chronic phase is hyperreactive to vasoconstrictors when arterial hypertrophy, which increases the responsiveness [11, 12], is fully established [13]. In order to assess whether the possible alterations in vascular reactivity are restricted to a specific agonist or, in contrast, affect the contractile machinery, receptor-mediated and nonspecific smooth muscle stimulants were used.

Materials and methods

Male Wistar rats initially weighing 120–150 g received a standard laboratory rat chow diet and tap water ad libitum throughout the study. Renal hypertension was induced by placing a silver clip (inside diameter 0.2 mm) around the left renal artery under ether anesthesia. Age-matched control animals were subjected to the same surgical manipulation without clipping. Sixteen weeks after the induction of hypertension, the rats were killed by decapitation and their aortas studied as described below.

Blood pressure was measured by the tail cuff method in unanesthetized rats. At least five determinations were made in each rat on the day before the experiment, and averaged to serve as the final blood pressure value. A systolic blood pressure of 150 mm Hg or higher was the criterion for hypertension.

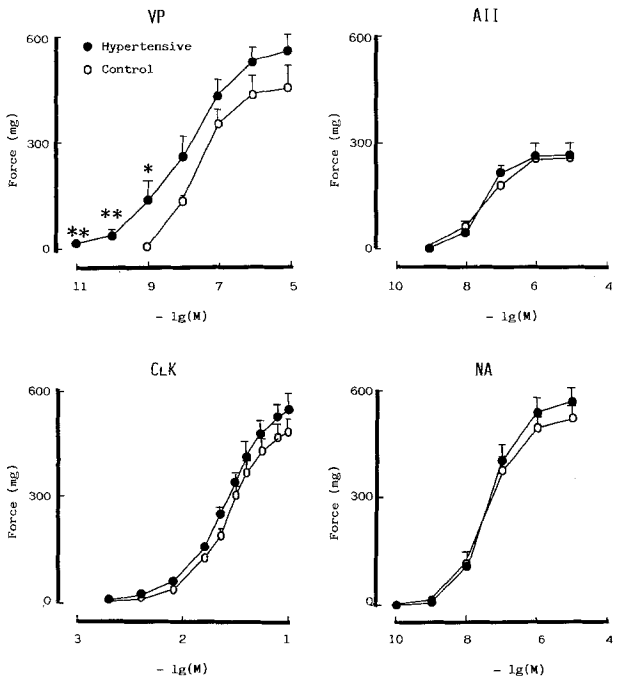
Thoracic aortas were excised and fat and connective tissue was removed, taking special care to leave the endothelium intact. Helical (0.3 × 2 cm) strips were cut and suspended in a standard muscle bath chamber in physiologic salt solution (PSS) of the following composition (in mM): NaCl, 118; KCl, 4.7; KH_2PO_4, 1.18; $MgSO_4 \cdot 7H_2O$, 1.17; $CaCl_2 \cdot 2H_2O$, 1.6; $NaHCO_3$, 14.9 and dextrose 5.5. The strips were maintained at 37 °C and aerated with 95 % O_2, 5 % CO_2. Vascular strips were attached to a force transducer (Leti graph 2000, Letica) for measurement of isometric contraction force. Strips were allowed to equilibrate for 60 min with a resting tension of 2 g prior to the start of the experiment. Cumulative dose-response curves to vasopressin (10^{-10}–10^{-5} M), angiotensin II (10^{-9}–10^{-5} M), KCl (8–80 mM) and noradrenaline (10^{-10}–10^{-5} M) were generated. Sufficient time (approximately 40 min) was allowed between each dose-response curve to facilitate washout of the drugs. Concentration of the drugs was increased once the contractile tension had reached a steady-state response to the prior concentration (approximately 3 min). Angiotensin II, noradrenaline-HCl and vasopressin were purchased from Sigma Chemical (St. Louis, MO, USA). Solutions were made fresh each day in PSS from a frozen aliquot.

Threshold (ED_{10}) sensitivity values were determined following logit transformation of normalized dose-response curves. Differences in systolic blood pressure and ED_{10} values between control and renal hypertensive rats were analyzed with the unpaired Student's t-test. A p-value of less than 0.05 was considered significant.

The systolic blood pressure of control and renovascular hypertensive rats was 131 ± 2 (mean ± SEM) and 190 ± 5 ($p < 0.001$), respectively. The dry weight of the strips (3 × 20 mm) from hypertensive rats was 15.2 ± 2 mg and that from the matched control was 12 ± 1.5 mg, with no significant difference between the two.

Results and discussion

Cumulative concentration effect curves were obtained for vasopressin (VP), angiotensin II (AII), noradrenaline



Contractile responses of aortic strips from chronic Goldblatt two kidney-one clip hypertensive (n = 6) and age-matched control (n = 6) rats to vasopressin (VP), angiotensin II (AII), KCl and noradrenaline (NA). Data are expressed as mean ± SEM. *$p < 0.05$; **$p < 0.01$.

(NA) and KCl to compare the responses of aortic strips from chronic renovascular hypertensive rats and from control rats (fig.). Helical strips of thoracic aorta from hypertensive rats were more sensitive to VP than were those from control rats. The concentration of VP necessary for a threshold response (ED_{10}) in the thoracic aorta from hypertensive rats ($0.99 \pm 0.38 \times 10^{-9}$ M) was significantly lower ($p < 0.01$) than that in the control rats ($3.68 \pm 0.18 \times 10^{-9}$ M). However, at high doses both vascular strips were equally responsive to VP. The differences at the lower doses were significant when the data were expressed both as a percentage and as the absolute increase in tension.

The responses to AII, NA and KCl were similar in both groups. AII-induced contraction was reduced in comparison to the other agonists in both groups of rats, as indicated by the decrease in maximal force generated, estimated from cumulative concentration effect curves.

The present study was primarily designed to test the hypothesis that rats with chronic Goldblatt two kidney-one clip hypertension have an enhanced vascular reactivity to vasoconstrictors. The results show an increased sensitivity to VP in the vascular smooth muscle from hypertensive rats. The mechanism(s) by which vascular sensitivity to VP is increased in this model of hypertension is unclear. However, an alteration in VP receptors may be involved, since this alteration is specific for this agonist. The increase in responsiveness at lower concentrations of VP suggests enhanced sensitivity of the vascular smooth muscle to circulating levels of VP, and hence could play a role in increasing the peripheral resistance in the chronic phase of this type of hypertension. The pathophysiological significance of VP in this model of hypertension has only been studied in the early phase. Various studies indicate that VP does not contribute to the development of this type of renovascular hypertension[14-17] to the same extent as in the volume-expanded hypertension models, in which VP plays an essential role[14-16].

In the present investigation the aortic smooth muscle from hypertensive rats showed similar responses to those of muscle from control rats to AII, NA and KCl. These results are compatible with previous reports on aortic strips in the early phase[9], and those on anesthetized rats in the early and chronic phases[18]. However, increased reactivity to vasoconstrictors has been reported in aortic[7,8] and other preparations[19].

We also observed a decrease in maximal force generated in response to AII, when compared with the other agonists, in both groups of rats. Similar findings have been reported in preparations from hypertensive and control rats, where resistance vessels are present[20,21]. Aging may explain this decreased vascular reactivity to AII, since a markedly reduced maximal response to AII with respect to NA was observed in aortic strips of 10-week-old normal rats, whereas a similar response to both vasoconstrictors was observed in 7-week-old normal rats[22].

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