Effect of dietary sodium supplementation, neonatal sympathectomy and structural vascular changes on the development of angiotensin II-induced hypertension
Prognostic value of blood pressure monitoring in kidney disease

PhD Thesis

Botond Csiky, MD

University Medical School of Pécs, Hungary
Nephrological Center and 2nd Department of Medicine

Tutor: Prof. Judit Nagy MD, DSc

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LIST OF ABBREVIATIONS

ABPM:	24-hour noninvasive ambulatory blood pressure monitoring
ACE:	angiotensin - converting enzyme
ACEI:	angiotensin - converting enzyme inhibitor
Ang II:	angiotensin II.
AVP:	arginine vasopressin
BP:	blood pressure
CCB:	calcium – channel blockers
ip:	inraperitoneally
MAP:	mean arterial pressure
NE:	norepinephrine
NS:	not significant
OD:	outer diameter of arteries
PE:	phenylephrine
SBP:	systolic blood pressure
SMA:	superior mesenteric artery
sc:	subcutaneously
WLR:	wall – to – lumen ratio (arteries)
wk:	week

2. SUMMARY

- The long-term administration of initially subpressor doses of angiotensin II (Ang II) to rats mimics the development of human renovascular and high-renin hypertension.
- In this animal model short-term administration of high-sodium diet appears to potentiate vasoconstrictor responses to Ang II by facilitating sympathetic neurotransmission, and long-term administration of high-sodium diet raises systolic blood pressure by potentiating the trophic vascular effects of Ang II. The interaction appears to be specific to Ang II and is occuring on the vascular level.
- The onset of hypertension in this model is preceded by a prehypertensive period characterized by potentiation of pressor and vasoconstrictor responses to Ang II itself (autopotentiation). Autopotentiation of vasoconstrictor responses appears to be due to an interaction between Ang II and the sympathetic nervous system, because it is prevented by sympathectomy. The dissociation of function and structure of the small arteries in the chronic stage of Ang II administration to sympathectomized rats suggests that structural vascular changes by themselves are insufficient to cause hypertension, but increased vascular reactivity or vasoconstrictor input is also needed.
- To investigate further the dissociation between hypertension and structural vascular changes in sympathectomized Ang II-treated rats, we extended the morphometric measurements to include all segments of the mesenteric vascular tree. Hypertension and sympathetic innervation appear to be contributing to the development of structural changes in large arteries of Ang II-treated rats because sympathectomy attenuated these changes. Structural changes in small

arteries, on the other hand, appear to be due to a direct trophic effect of Ang II.

- Ang II-induced hypertension and structural vasular changes are dose- and time-dependent and synergistically enhanced by dietary sodium supplementation.
- Primary IgA nephropathy is the most common type of primary glomerulonephritis, in which hypertension is a recognized marker of poor prognosis. Hypertension in IgA nephropathy is a typical example for secondary human hypertension. Ang II seeems to play an important role in the hypertension developing in IgA nephropathy patients. According to the results of 24 hour blood pressure monitoring, there is no diurnal blood pressure variation in most of the hypertensive IgA nephropathy patients. Antihypertensive treatment (angiotensin-converting enzyme inhibitors and calcium-channel blockers) has better effect on day-time than on night-time hypertension. The lack of the circadian blood pressure rhythm and „white-coat" hypertension seems to accelerate the progression of IgA nephropathy.

3. INTRODUCTION

Most people will develop hypertension during their lifetime (1). Despite the great progress which has been made in hypertension research during the past decades, hypertension remains a common and serious health problem with many unsolved questions, contributing in a major way to the most common causes of morbidity and mortality in developed societies (2). Although awareness, treatment and control of hypertension have increased significantly in the past 10 years in the United States (where the National Health and Nutrition Examination Surveys, the NHANES studies have been conducted), only about two-third of the patients are aware of their high blood pressure, only about one-half of patients are treated and only one-quarter have their hypertension under adequate control (3, 4). We have no reasons to believe that this situation is significantly better in other countries. These improvements in the recognition and treatment of hypertension have played a role in the major reductions in cardiovascular disease seen in the United States over the past decades (5). However, it could be observed a lack of control of two major conditions which are often the consequence of uncontrolled hypertension: end stage renal disease and congestive heart failure (6). The treatment of hypertension can delay the appearance of these disorders. It is possible, that prevention can be achieved with more agressive treatment and with more effective agents, such as angiotensin-converting enzyme inhibitors (ACEIs).

It has been postulated that angiotensin II (Ang II) plays a pathogenetic role in several forms of human hypertension. Circulating levels of Ang II are increased in renovascular hypertension, in several forms of renoparenchymal hypertension and in about one third of

patients with borderline essential hypertension (7, 8). Ang II, on the other hand, seems to play an important role in almost every form of human hypertension: in the so-called normal renin and low-renin forms of hypertension, increased production of Ang II in vascular tissue and myocardium may contribute to the development of the disease (9).

The long-term administration of initially subpressor doses of Ang II to rats mimics the development of human renovascular and high-renin (Ang II) essential hypertension (10, 11). It may also be a model of other high-renin secondary hypertension (e.g. renoparenchymal hypertension). The onset of hypertension in this model is preceded by a prehypertensive period characterized by trophic stimulation of vascular muscle and potentiation of pressor and vasoconstrictor responses to Ang II itself (autopotentiation) (12). This is followed by the onset of hypertension and the development of structural vascular changes (13).

The above described animal model of hypertension is a very useful tool in the study of several factors influencing the development of Ang II-induced hypertension: the effect of high-sodium diet, the sympathetic nervous system, structural vascular changes, the dose and time of the administered Ang II.

3.1. *The effect of dietary sodium supplementation on Ang II-induced hypertension*

The acceleration and exacerbation of the hypertensive process by high-sodium diet are well-known phenomena, but the underlying mechanisms are poorly understood (14). There is experimental evidence, that the long-term administration of moderately high-sodium diet (2% NaCl chow or 1% saline) to rats may lead to increased vascular reactivity to a number of agonists without producing hypertension (15, 16, 17). This

suggests that the interaction between vasoconstrictor agonists and dietary sodium supplementation may take place in part on the vascular level. There is also evidence, that high-sodium diet increases pressor responses to Ang II and norepinephrine (NE) in human subjets (18), but changes in pressor responses cannot be easily equated with changes in vascular reactivity. These hemodynamic effects of high sodium intake may be reinforced through facilitation of sympathetic activity at nerve endings (19, 20).

Salt and water retention has not been found in the above mentioned rat model of hypertension (10, 11).

3.2. *Effect of neonatal sympathectomy on development of Ang II-induced hypertension*

The possibility of interaction between the renin-angiotensin system and the sympathetic nervous system has generated a great deal of investigation, because both systems may be simultaneously activated in some forms of human hypertension (21). Ang II was shown to increase sympathetic vasoconstrictor tone by acting directly on the central nervous system (12) and by altering the uptake and release of NE at nerve terminals (22). Ang II may also potentiate vascular responses to NE (23). However, most of these observations have been based on acute experiments, often using pharmaceutical doses of Ang II. It is not clear whether the findings of acute experiments can be extrapolated to situations of chronic stimulation of the renin-angiotensin system, the experimental reproduction of which is the long-term administration of Ang II (10).

When Ang II is administered in doses that have an immediate pressor effect, the renal vasoconstrictor action of the agonist and the

resulting salt and water retention overshadow other potential hypertension-producing mechanisms (10, 11). Under these circumstances, an intact sympathetic nervous system is not required for the development of hypertension. In uninephrectomized rats given pressor doses of Ang II, denervation of the remaining kidney did not prevent or attenuate the hypertension (24). When pressor doses of Ang II were infused into dogs, the concomitant administration of guanethidine was equally ineffective (25). Similarly, sympathectomy with 6-hydroxydopamine did not prevent hypertension in Wistar-Kyoto and spontaneously hypertensive rats treated with pressor doses of Ang II (26).

When Ang II is administered in small or initially subpressor doses, a different pathophysiological picture emerges (10, 11). The onset of hypertension is gradual and progressive; salt and water retention cannot be detected.

3.3. *Effect of neonatal sympathectomy on the development of structural vascular changes in Ang II-treated rats*

The hallmark of chronic hypertension is the development of structural vascular changes, and an increase in the wall-to-lumen ratio (WLR) of the arteries is the predominant lesion (27, 28, 29, 30). An increase in WLR does not necessarily indicate that vessel wall area has increased (hypertophy) but could be due to a decrease in the lumen (remodeling). In the genetic forms of hypertension, including human essential hypertension, remodeling of resistance arteries appears to play an important role. In the rat models of experimental hypertension, on the other hand, much of the altered structure is due to vascular growth associated with hypertrophy of vascular smooth muscle cells. Whether

structural vascular changes are primary or secondary to blood pressure elevation remains an unsettled issue. In the majority of studies of hypertension, a direct relationship between blood pressure elevation and WLR has been found, but discrepanties also exist (31). It has been proposed that trophic stimulation of vascular muscle alone might be sufficient to induce structural vascular changes and, thereby, initiate the hypertensive process. Ang II has been a prime candidate for such a trophic factor (32). The trophic effects of Ang II may be reinforced by concomitant activation of the sympathetic nervous system, which could have trophic properties of its own (32). Previous investigation of the administration of Ang II to rats (13, 33) revealed medial thickening due to hypertrophy of large and small mesenteric arteries. Medial thickening of large arteries was not prevented by antihypertensive therapy with hydralazine, suggesting that pressure-independent mechanisms were also operating (33).

3.4. *Structural vascular changes in hypertension – role of Ang II, dietary sodium supplementation, blood pressure and time*

As structural vascular changes are the hallmark of chronic hypertension, and increased WLR of resistance arteries is the predominant lesion (27, 28, 29, 30), Ang II itself, dietary sodium supplementation, blood pressure and time may play an important role in the development of these structural vascular changes. The increase in relative „thickness" of resistance arteries is responsible for the „amplifier" property of the arterial circulation in hypertension, which functionally manifests itself as a pressor or vasoconstrictor hyperresponsiveness. Some studies have raised the question of whether a trophic stimulus alone may be sufficient to induce structural vascular changes and thereby initiate the

hypertensive process (32). High-sodium diet may play an important role in the development of structural vascular changes induced by a certain trophic stimulus.

Preliminary data in our laboratory indicated that the development of Ang II-induced hypertension in rats is both dose- and time-dependent. By time-dependence we mean the phenomenon whereby one half of an effective dose of Ang II may take twice as long as the full dose or longer to produce hypertension and structural vascular changes. This is an important concept because it suggests that a stimulus that is undetectable at any one point in time, when applied long enough, may lead to hypertension.

3.5. *Hypertension and progression of the kidney disease in patients with IgA nephropathy*

Primary IgA nephropathy is the most common type of primary glomerulonephritis worldwide (34, 35). Hypertension is a recognized marker of poor prognosis in IgA nephropathy (34, 36-40). Hypertension in IgA nephropathy is a typical example for secondary human hypertension. Experimental evidence has shown that ACEI treatment leads to a significant attenuation of proteinuria in patients with chronic glomerulonephritis (41). ACEI treatment has been demonstrated to reduce urinary protein excretion, to attenuate progression and to improve prognosis of the kidney disease (42, 43). Deletion polymorphism in the angiotensin-converting enzyme (ACE) gene has been shown to be a risk factor for progression to chronic renal failure in IgA nephropathy (44-46). Moreover, the deletion polymorphism predicts the therapeutic efficacy of ACE inhibition on proteinuria and, potentially, on progressive deterioration of renal function (46). The above mentioned

findings strongly suggest that Ang II has a crucial role in the development of hypertension in IgA nephropathy, in the progression of the kidney disease and on the efficacy of different antihypertensive medications in these patients.

The increasing availability of 24-hour noninvasive ambulatory blood pressure monitoring (ABPM) has shifted interest in blood pressure measurement from the doctor's office to the entire 24-hour period. As hypertension has an important role in the progression of the kidney disease in IgA nephropathy, it is important to study the blood pressure of these patients in detail. The use of ABPM makes possible the noninvasive blood pressure measurement during 24 hours in the patients environment. It allows the diagnosis of „white-coat hypertension", in which blood pressure values are high on office measurements but normal on ambulatory monitoring. It can be utilized to identify „dippers" and „non-dippers" among patients (47-49). Routine office blood pressure recordings correlate poorly with left ventricular mass, a sign of early target organ damage. At the same time ambulatory blood pressure shows good correlation with left ventricular mass (50-52). „Non-dippers" seem to be at greater cardiovascular risk than „dippers". Patients with white-coat hypertension develop renal impairment and left ventricular hypertrophy. White-coat hypertensive subjects seem to be at greater cardiovascular risk than normotensive subjects. Cardiac involvement seems to precede glomerular damage in the early stage of hypertension (53-55). Similarly, IgA nephropathy patients with „white-coat" hypertension and/or nocturnal hypertension may be at greater risk for deterioration of kidney functions, than normotensive IgA nephropathy patients.

4. AIMS OF THE STUDIES

4.1. *The effect of dietary sodium supplementation on Ang II-induced hypertension*

The aim of this study was to investigate the effects of dietary sodium supplementation on the development of hypertension in this model. We were interested to test whether high-sodium diet enhanced the vasoconstrictor responses to Ang II itself and whether it accelerated the development of structural vascular changes and of hypertension. With regard to the former, we hypothesized that facilitation of peripheral sympathetic activity on the vascular level may be the basis of the synergistic interaction between salt and Ang II.

4.2. *Effect of neonatal sympathectomy on development of Ang II-induced hypertension*

The purpose of these experiments was to investigate the effect of neonatal sympathectomy on the evolution of hypertension induced by small doses of Ang II. Measurements were performed in the developmental stage of hypertension to test whether sympathectomy prevented the autopotentiation of vasoconstrictor responses to Ang II and in the chronic, established phase of hypertension to test whether the antihypertensive effect of sympathectomy, if any, was related or not to the prevention of structural vascular changes.

4.3. *Effect of neonatal sympathectomy on the development of structural vascular changes in Ang II-treated rats*

This study is an extesion of the previous one, in which we studied the structural changes in the small mesenteric arteries. The purpose of the present study was to extend the morphometric measurements to all segments of the mesenteric arterial tree, including the large arteries. Investigation of the large arteries permits to determine whether structural vascular changes induced by Ang II are due to remodeling, hypertrophy or hyperplasia.

4.4. *Structural vascular changes in hypertension – role of Ang II, dietary sodium supplementation, blood pressure and time*

The first aim of this investigation was to provide experimental data for the dose and time dependence of Ang II-induced hypertension. The second aim of this investigation was to explore the relationship between the onset of hypertension and the development of structural vascular changes in Ang II-treated rats. The effect of high-sodium diet on the rate of onset and extent of hypertension and structural vascular changes in Ang II-treated rats was also investigated.

4.5. *Hypertension and progression of the kidney disease in patients with IgA nephropathy*

The aim of this study was to investigate the prevalence of „white-coat hypertension", the diurnal blood pressure rhythm and the effectiveness of antihypertensive drug therapy in IgA nephropathy patients. The effect of „white-coat hypertension", lost or preserved diurnal blood pressure variation and the effect of antihypertensive therapy on the progression of IgA nephropathy was also studied.

5. METHODS AND RESULTS

5.1. *The effect of dietary sodium supplementation on Ang II-induced hypertension*

5.1.1. *Methods*

Design of the experiments. The experiments were performed after 7-10 days and 12 weeks of Ang II or phenylephrine (PE) administration to rats. The agonists were administered in initially subpressor doses. Because of the progressive pressor action of Ang II, the administered dose of Ang II was less during the 12-week than during the 7-to 10-day experiments. Similarly, dietary sodium supplementation was reduced from 4% NaCl during the short-term experiments to 2% NaCl during the 12-wk experiments to prevent the development of severe hypertension.

Preparation of rats. Pathogen-free, male Sprague-Dawley rats were used throughout these experiments. Osmotic minipumps (Alza, Palo Alto, CA, USA) were used to deliver Ang II or PE. The dose of Ang II (Sigma Chemicals, St. Louis, MO, USA) was 200 $ng \cdot kg^{-1} \cdot min^{-1}$ ip and 50 $ng \cdot kg^{-1} \cdot min^{-1}$ sc for the 7-10 day and 12 week experiments, respectively. PE (Sigma Chemicals) was infused at 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$. Control rats were fitted with empty minipumps. The rats had free access to tap water and received either normal, intermediate-sodium or high-sodium diet containing 125, 340 and 680 mmol/kg, or 0.7, 2, or 4% NaCl, respectively. The diets were matched for other ingredients. The rats received the following treatment: 200 $ng \cdot kg^{-1} \cdot min^{-1}$ ip Ang II (n=12), 4% NaCl diet (n=11), Ang II + 4% NaCl diet (n=7), and 0.7% NaCl diet (controls) (n=15). Additional rats received 50 $ng \cdot kg^{-1} \cdot min^{-1}$ sc Ang II (n=8), 2% NaCl diet (n=9), Ang II + 2% NaCl diet (n=6), or 0.7% NaCl

diet (controls) (n=10) for 12 weeks. To test the specificity of the interaction between salt and Ang II, experiments were also carried out after administration of PE to rats on normal- or high-sodium diet.

Systolic blood pressure (SBP) was measured in restrained awake rats by the tail-cuff method between 8 AM and 11 AM. During the 7-10 day experiments, these measurements were obtained twice, 1-3 days prior to the final experiment, and averaged. For the 12-week experiments, the SBP of rats was measured on at least two occasions prior to the insertion of the minipump to rule out spontaneous hypertension (SBP>130 mmHg). After the insertion of the minipump, the SBP of rats was measured weekly for 4 weeks and then every 2 weeks for the rest of the experiment. On each occasion, SBP was recorded three or more times, and the measurements were averaged.

Mesenteric vascular responses. The in-situ blood-perfused mesentery preparation developed by Jackson and Campbell was used (56). Rats were anesthetized with chloralose (75 mg/kg iv) (Sigma Chemicals) and placed on a preheated operation table. Body temprature monitored through an oral thermometer was kept at 37±0.5 °C throughout the experiments. The rats were allowed to breath spontaneously through a tracheostomy tube. The abdominal aorta downstream from the renal arteries and the superior mesenteric artery (SMA) were cannulated, and the SMA circulation was pump-perfused (Minipuls II; Gilson Medical Electronics, Middleton, WI, USA) at 4.0 ml/min with arterial blood from the rat's aorta. Arterial pressure through a cannulated carotid artery and perfusion pressure through a T-tube arrangement placed in the perfusion line distal to the pump were measured. After stabilisation of the perfusion pressure, incremental doses of Ang II (12 to 72 ng), norepinephrine (NE, 60 to 720 ng), and arginine vasopressin (AVP, 0.45 to 4.50 mU) were injected into the pump tubing upstream to the pump,

and the resulting perfusion pressure was recorded. Between injections, perfusion pressure was allowed to return to baseline. After the injection of the agonists, vasoconstrictor responses to periarterial nerve stimulation were measured. Bipolar platinum electrodes were placed around the SMA 5 mm distal to the cannulation point. Vasoconstrictor responses were obtained at 3-min intervals by stimulating for 20 s at 3, 5, 7, and 9 Hz. Results were expressed as change in perfusion pressure, because at constant blood flow, perfusion pressure is a direct measure of vascular resistance.

In situ tissue fixation. The rats were anesthetized with chloralose. After systemic anticoagulation (heparin, 1.000 IU iv) and vasodilatation (papaverine, 500 μg/rat iv), the mesenteric and left renal cicrulation of rats was pump-perfused retrograde through the abdominal aorta with aerated Krebs-Ringer bicarbonate solution. The inferior vena cava was cut open to allow free circulation of the perfusate. Perfusion pressure in the aorta was adjusted to 55-60 mmHg and kept there by adjusting the pump flow rate. The pressure range of 55-60 mmHg was chosen, because we have found previously that after maximal systemic vasodilation, mean arterial pressure falls to this level in rats treated with Ang II and in control rats (12). The mesenteric and left renal beds were perfused sequentially with Krebs-Ringer solution, a fixative containing glutaraldehyde and phosphate buffer. At the end of perfusion, the entire small intestine and mesenteric vascular arcade were removed and stored in formalin.

Morphometric measurements. Measurements were restricted to the small intestine and mesentery. Intestinal segments were dehydrated through graded series of ethanol, infiltrated and embedded in paraffin in a manner to permit cross-sectional cuts of mesenteric arteries. Four micrometer thick sections were stained with hematoxylin and eosin. Two

criteria were used to select arteries for morphometric measurements. First, by inspection, the thickness of the vessel wall had to be uniform throughout its circumference. Second, only vessels with a long-to-short axis ratio of <1.30 were measured, thus excluding vessels cut at greater than 40 angular degrees from cross-section. Two categories of resistance arteries, one with external diameter of 50-100 μm and the other with external diameter of 100-150 μm, were investigated. Morphometric measurements of external and lumen diameter along the long and short axis of cross-sectionally cut arteries were obtained under x450 magnification with a calibrated filar micrometer. The two measurements of external and lumen diameter were averaged. Measurements were made on a minimum of three cross-sectionally cut arteries (average 7.2). Wall thickness and WLR and the mean of each parameter was calculated for each rat.

Statistical analysis. Group comparisons were made by one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls range test for individual group comparisons. Repeated measures ANOVA (one factor within, one factor between) was used to compare weekly blood pressure values and mesenteric vasoconstrictor responses to agonists and nerve stimulation in the variously treated groups of rats and in control rats. Statistical analysis of vessel dimensions was restricted to WLR ratios. WLRs are relatively unchanged despite differences in lumen diameter as long as a small range of diameters is being measured and are, therefore, less affected by sampling bias than measurements of vessel diameters or wall thickness. WLR values in the five groups of variously treated rats were compared with those of control rats by two-factor ANOVA (factor 1, treatment; factor 2, small and intermediate size arteries) followed by preplanned contrasts (Superanova). Null hypotheses were rejected at $P<0.05$.

5.1.2. *Results*

Mesenteric vasoconstrictor responses. After 7-10 days of treatment, there were no significant changes in systolic blood pressure in any of the groups.

In Ang II treated rats, mesenteric vasoconstrictor responses to Ang II were increased (autopotentiation). High-sodium diet increased vasoconstrictor responses to Ang II ($P<0.01$) and nerve stimulation ($P<0.02$), and in combination with Ang II treatment further potentiated vasoconstrictor responses to Ang II (synergism, Fig. 1). Vasoconstrictor responses to NE and AVP were unchanged in all treatment groups compared with controls.

Pressor responses. Over the 12 week period, hypertension developed only in rats receiving the combined treatment of Ang II and high-sodium diet (Fig. 2).

Structural vascular changes. After 12 wk of treatment, Ang II increased W/L of small resistance arteries by 11% ($P<0.05$) without a significant rise in systolic blood pressure. Ang II and 2% NaCl diet in combination raised systolic blood pressure by 36 mmHg ($P<0.01$, Fig. 2) and increased small artery WLR by 28% ($P<0.001$, Fig. 3) compared with values obtained in control rats. In these rats, WLR of not only the small arteries but also of the intermediate size arteries was increased (Fig. 3).

To test the specificity of the interaction between Ang II and high-sodium diet, all the experiments were repeated during phenylephrine (10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ sc) treatment of rats. Phenylephrine by itself or in combination with high-sodium diet had no effect on the measured parameters.

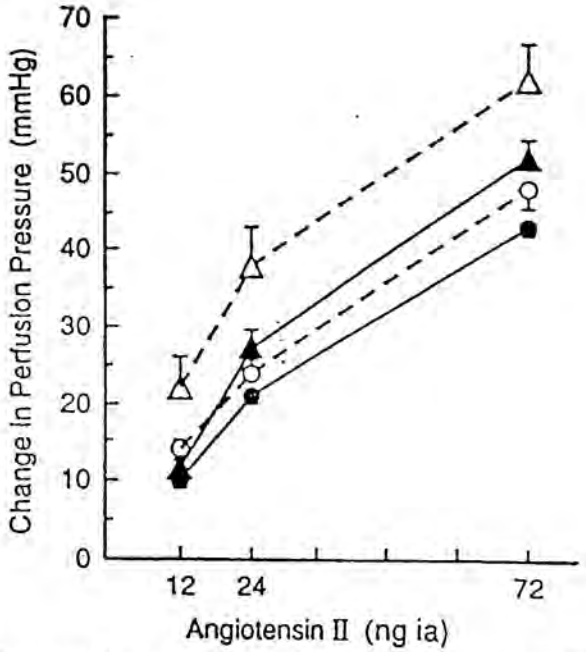

Fig. 1. Vasoconstrictor responses to ANG II (means ± SE) in the pump-perfused mesenteric circulation of rats after 7–10 days of the following treatments: 200 ng·kg^{-1}·min^{-1} ip ANG II (○; $n = 12$, $P < 0.05$), 4% NaCl diet (▲; $n = 11$, $P < 0.01$), 200 ng·kg^{-1}·min^{-1} ANG II ip + 4% NaCl diet (△; $n = 17$, $P < 0.002$), or 0.7% NaCl diet (control, ●; $n = 15$).

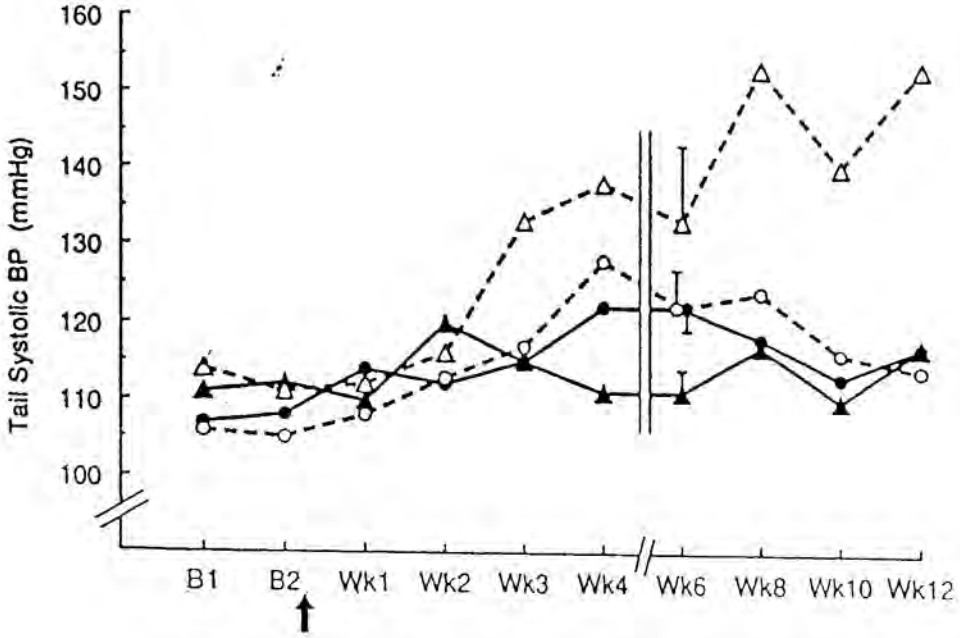

Fig. 2. Tail systolic blood pressure (SBP, means ± SE) of rats treated with 50 ng·kg^{-1}·min^{-1} sc ANG II (○, $n = 8$), 2% NaCl diet (▲, $n = 9$), 50 ng·kg^{-1}·min^{-1} sc ANG II + 2% NaCl diet (△; $n = 6$, $P < 0.01$), or 0.7% NaCl diet (control, ●; $n = 10$). Arrowhead, start of treatments. B1 and B2, baseline measurement 1 and 2, respectively.

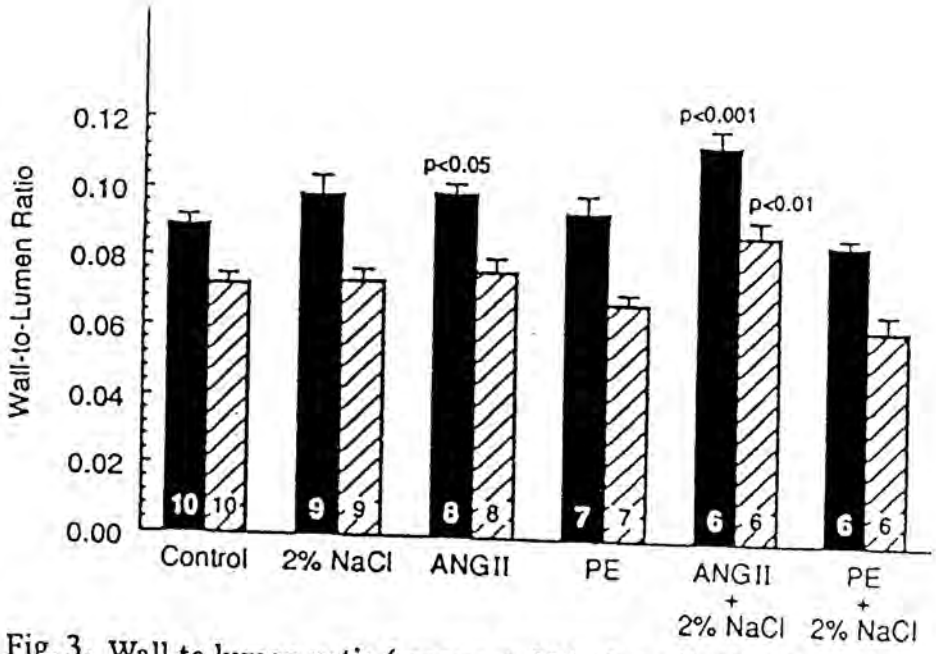

Fig. 3. Wall-to-lumen ratio (means ± SE) of small (50–100 μm OD, solid bars) and intermediate size (100–150 μm OD, hatched bars) mesenteric resistance arteries of rats on the various 12-wk treatment regimens; no. of rats is shown at base of each bar.

5.1.3. *Summary*

Short-term administration of high-sodium diet appears to potentiate vasoconstrictor responses to Ang II by facilitating sympathetic neurotransmission, and long-term administration of high-sodium diet raises SBP by potentiating the trophic vascular effects of Ang II. The interaction appears to be specific to Ang II and is occuring on the vascular level.

5.2. *Effect of neonatal sympathectomy on development of Ang II-induced hypertension*

5.2.1. *Methods*

Preparation of rats. To achieve sympathectomy, we used the technique of neonatal guanethidine injections and bilateral adrenal medullectomy (57). These interventions achieve a virtual absence of peripheral sympathetic innervation in adult rats.

Neonatally sympathectomized and sham-sympathectomized male Sprague-Dawley rats rats received 100 or 200 $ng \cdot kg^{-1} \cdot min^{-1}$ Ang II ip for 7-10 days or 200 $ng \cdot kg^{-1} \cdot min^{-1}$ Ang II sc for 4 weeks. Sham-treated sympathectomized and sham-sympathectomized rats were controls.

Mesenteric vascular responses and in situ fixation. Vasoconstrictor responses to Ang II, NE, AVP and periarterial nerve stimulation were measured in the mesentery of rats, and thereafter, in the chronically treated rats, mesenteric resistance arteries were fixed in situ for morphometric measurements.

Morphometric measurements. A narrow range of small resistance arteries, with external diameter between 100 and 150 μm, were investigated. Measurements were made on a minimum of five cross-sectionally cut arteries (average 7.9).

The methods are described in details in the previous chapter (Chapter 5.1.1.).

Statistical analysis. Group means were compared by one-way analysis of variance (ANOVA; F test). Weekly systolic blood pressure in a given group of rats was analyzed by repeated measures ANOVA (1 factor within, 0 between). Repeated measures ANOVA (1 factor within, 1 between) was used to compare weekly systolic blood pressure and dose-response curves of Ang II-treated and sham-treated (control) rats.

WLR in the four groups of chronically treated rats were compared by one-way ANOVA (F-test). Fisher's protected least significant difference post hoc test was applied to the mean differences after ANOVA. Null hypotheses were rejected at $P<0.05$.

5.2.2. Results

Pressor and mesenteric vasoconstrictor responses. In Ang II-treated sham-sympathectomized rats tail systolic blood pressure was unchanged after 7-10 days and increased by 23 mmHg at week 4 ($P<0.001$, Fig. 4). In these rats vasoconstrictor responses were selectively increased to Ang II (autopotentiation, $P=0.026$) and nerve stimulation ($P=0.031$) at 7-10 days (Fig. 5) and nonselectively increased to all stimuli at 4 weeks ($P<0.05$ to $P<0.01$, Fig. 6).

In Ang II-treated sympathectomized rats, there were no changes in systolic blood pressure (Fig. 4) or vasoconstrictor responses at either 7-

10 days (Fig. 7) or 4 weeks (Fig. 8) compared to sham-treated sympathectomized rats.

Compared with sham-sympathectomized controls (Fig. 6), sympathectomized control rats had exaggerated vasoconstrictor responses to NE (denervation hypersensitivity) and markedly reduced vasoconstrictor responses to nerve stimulation (denervation).

Structural vascular changes. In Ang II-treated sham-sympathectomized rats after 4 weeks, the WLR of resistance arteries was increased ($P<0.02$, Table 1). In Ang II-treated sympathectomized rats there were no changes in systolic blood pressure or vasoconstrictor responses, but structural vascular changes developed to the same extent as in sham-sympathectomized Ang II-treated rats (Table 1).

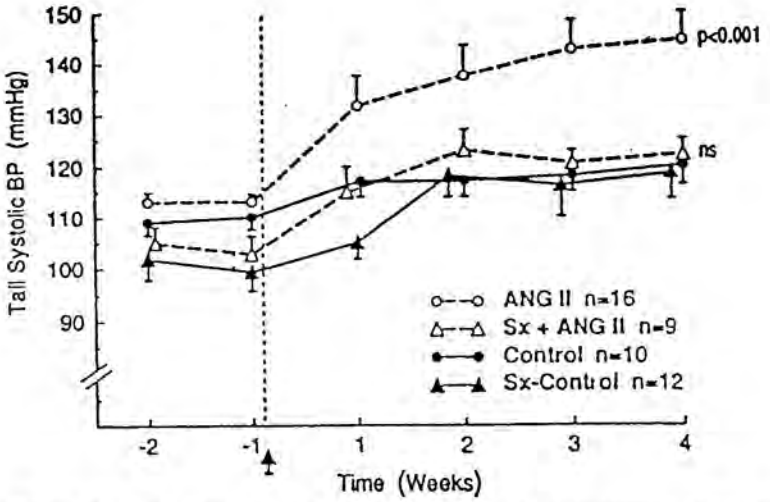

Fig. 4. Weekly tail systolic blood pressure (BP; means ± SE) of sympathectomized (Sx, triangles) and sham-Sx (circles) ANG II-treated (200 $ng \cdot kg^{-1} \cdot min^{-1}$ sc; dashed lines) and sham-treated (control, solid lines) rats. $P < 0.001$ compared with sham-Sx controls (filled circles). *n*, no. of rats.

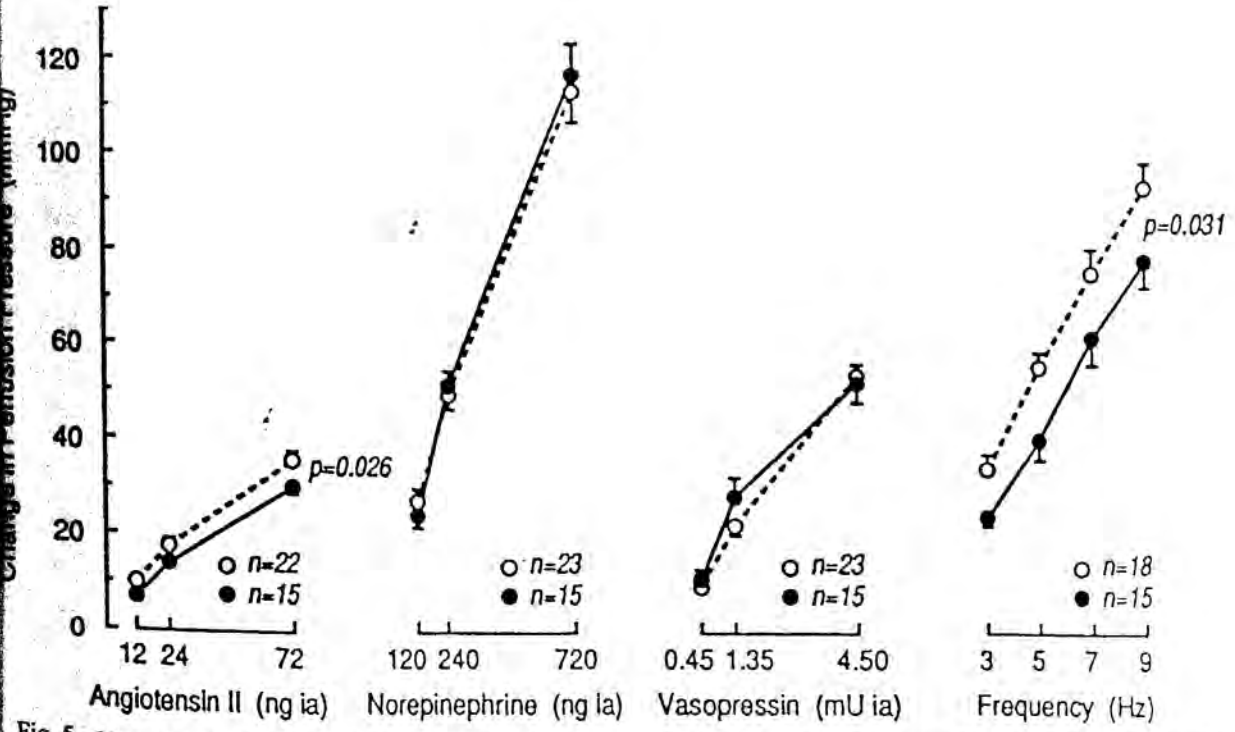

Fig. 5. Vasoconstrictor responses in pump-perfused mesenteric circulation of sham-Sx rats treated with ANG II (100 or 200 $ng \cdot kg^{-1} \cdot min^{-1}$ ip) for 7–10 days (open circles, dashed lines) or sham-treated rats (filled circles, solid lines). ia, Intra-arterial; *n*, no. of rats.

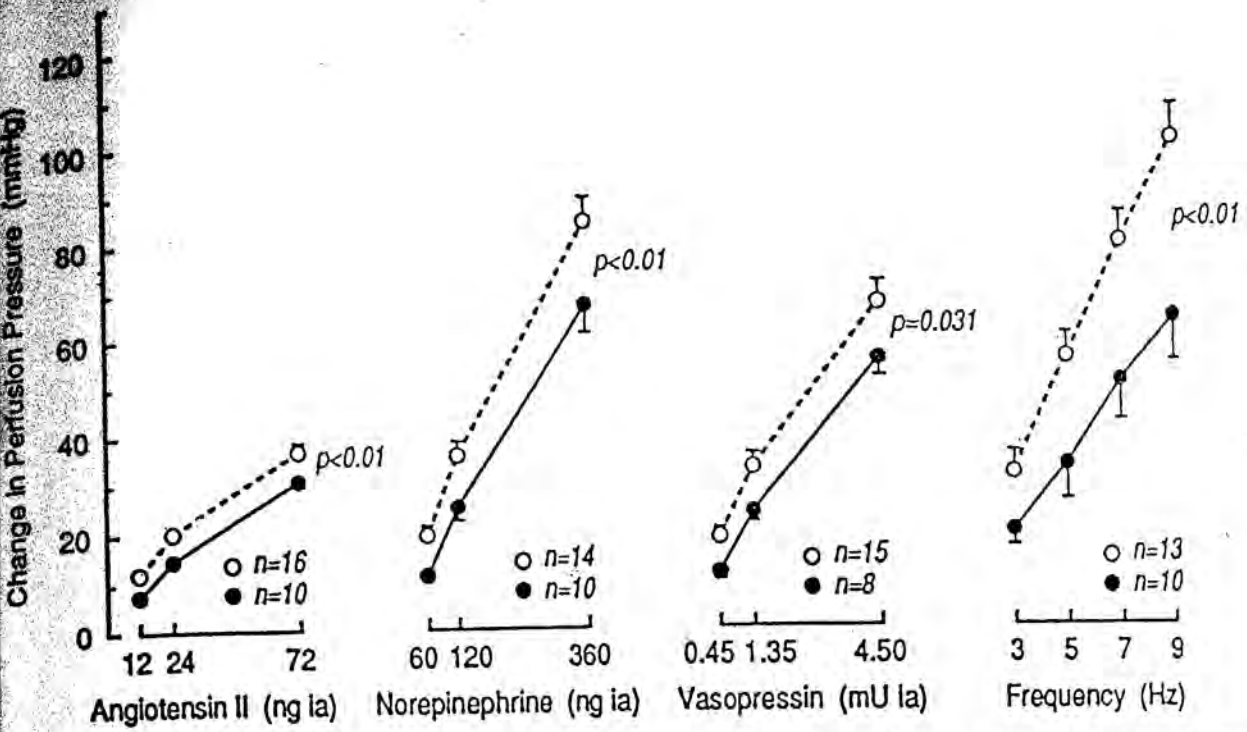

Fig. 6. Vasoconstrictor responses in pump-perfused mesenteric circulation of sham-Sx rats treated with ANG II (200 $ng \cdot kg^{-1} \cdot min^{-1}$ sc) for 4 wk (open circles, dashed lines) or sham-treated rats (filled circles, solid lines). *n*, no. of rats.

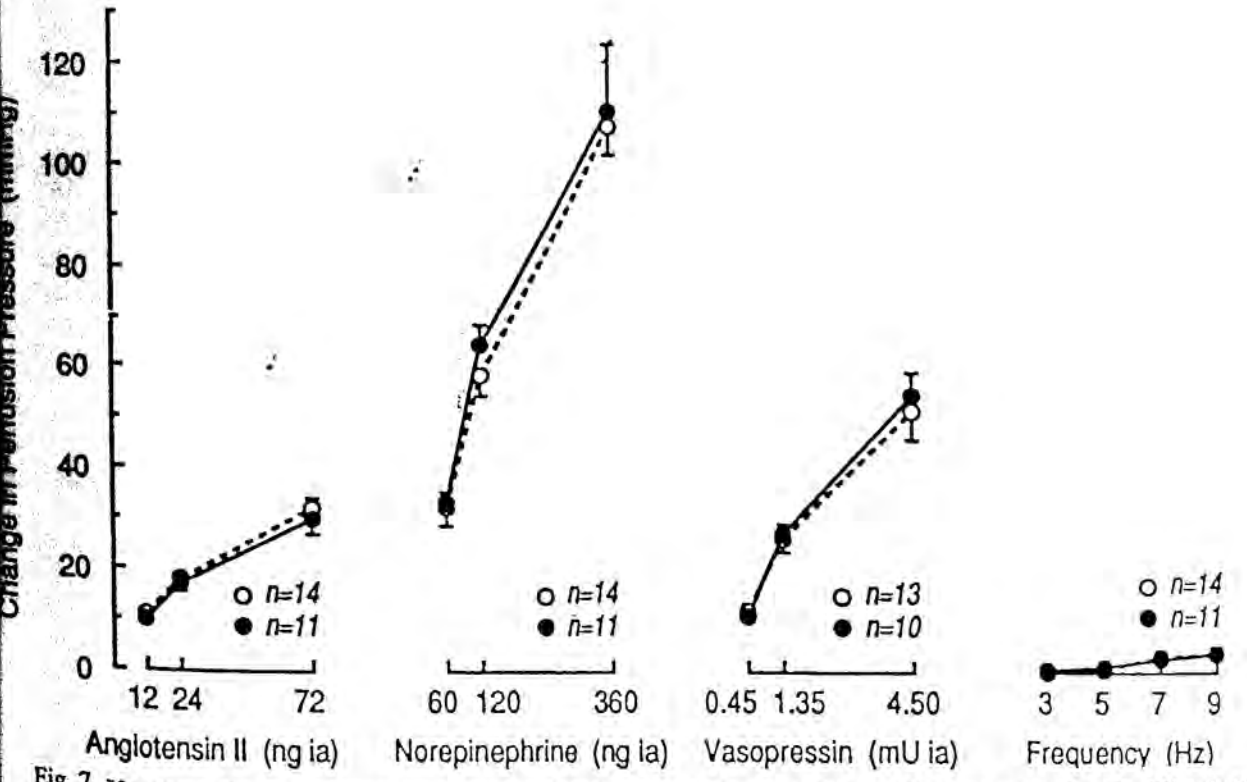

Fig. 7. Vasoconstrictor responses in pump-perfused mesenteric circulation of Sx rats treated with ANG II (200 $ng \cdot kg^{-1} \cdot min^{-1}$ ip) for 7–10 days (open circles, dashed lines) or sham-treated rats (filled circles, solid lines). *n*, no. of rats.

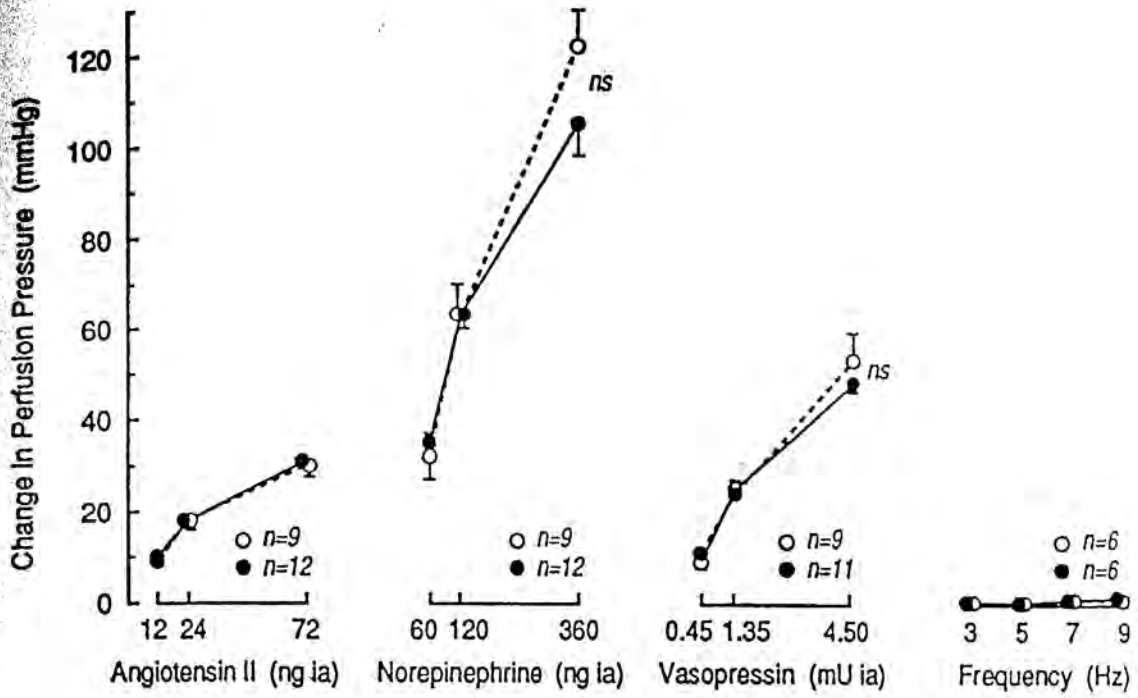

Fig. 8. Vasoconstrictor responses in pump-perfused mesenteric circulation of Sx rats treated with ANG II (200 $ng \cdot kg^{-1} \cdot min^{-1}$ sc) for 4 wk (open circles, dashed lines) or sham-treated rats (filled circles, solid lines). *n*, no. of rats.

Table 1. *Morphometry of resistance arteries in sham-sympathectomized and sympathectomized ANG II-treated and control rats*

	Sham-Sympathectomized		Sympathectomized	
	Control	ANG II	Control	ANG II
n	8	11	6	8
Vessel diameter, μm	114 ± 2	132 ± 5	118 ± 2	135 ± 5
Lumen diameter, μm	101 ± 2	113 ± 4	104 ± 2	116 ± 4
Wall thickness, μm	7.0 ± 0.1	9.3 ± 0.5	6.8 ± 0.3	9.8 ± 0.6
Wall-to-lumen ratio	0.070 ± 0.003	0.082 ± 0.004*	0.065 ± 0.002	0.084 ± 0.003†

Parameter values are means ± SE; *n*, no. of rats. ANG II (200 $ng \cdot kg^{-1} \cdot min^{-1}$ sc) was administered for 4 wk. *$P < 0.02$, †$P < 0.002$ in comparison with controls.

5.2.3. *Summary*

In rats treated with Ang II for 7-10 days, sympathectomy prevented autopotentiation, and in rats treated with Ang II for 4 weeks, sympathectomy prevented the exaggerated vasoconstrictor responses to Ang II, NE and AVP. Sympathectomy did not prevent or attenuate the increase in the WLR of small arteries that occured in hypertensive sham-sympathectomized Ang II-treated rats.

5.3. *Effect of neonatal sympathectomy on the development of structural vascular changes in Ang II-treated rats*

5.3.1. *Methods*

The *preparation of rats*, method of sympathectomy, blood pressure measurement and in-situ tissue fixation has been described in details in the previous chapters (Chapter 5.1.1. and 5.2.1.).

Morphometric measurements. Morphometric measurement of small mesenteric arteries has also been described in the previous chapters (Chapter 5.1.1. and 5.2.1.). In the present study, two categories of small resistance arteries, with external diameters in the ranges 50-100 (small) and 100-150 μm (intermediate size), were investigated.

In this study morphometric measurements were extended to the large mesenteric arteries: two vascular arcades with a Y configuration of the first- and second-order branches of the superior mesenteric artery were excised. The arteries were dehydrated through graded series of ethanol, infiltrated and embedded in epoxy. Cross-sectional cuts, 0.5 μm thick, of the arteries were made. The sections were stained with methylene blue. A video camera (Cell Analysis Systems, Inc., Lombard,

Illinois, USA) connected to an image analysis processor (NIH Image 1.54, public domain software) and a microcomputer was used to measure vessel and lumen diameters, wall thickness, and medial wall and lumen areas. WLR and medial: luminal area ratio were calculated. For a given rat, measurements taken for first- and second-order arteries, respectively, were averaged. Remodeling and growth indices of first- and second-order arteries of Ang II-treated rats were calculated according to the formulae published by Heagerty et al (29). Remodeling index was defined as the percentage of the observed difference in lumen diameter of hypertensive and normotensive arteries that could be accounted for by remodeling of the normotensive artery. A remodeling index of 100% indicated that the difference between the WLR of arteries in hypertensive and normotensive rats were entirely due to a change in lumen diameter. A remodeling index greater than 100% indicated that vascular muscle growth accounts in part for increased WLR of arteries in hypertensive rats. The growth index was an estimate of the magnitude of this growth.

To count the number of nuclei per unit length of vessel wall, first-order arteries were embedded in paraffin, stained with hematoxylin and eosin, and sectioned along their longitudinal axis (58). Using this sectioning, the nuclei of vascular muscle were cut transversely. The number of nuclei was counted under x450 magnification over 1.2-2.5 cm of vessel wall with calibrated filar micrometer. Results were expressed as number of nuclei/100 μm length.

Statistical analysis. Two-way analysis of variance (factor 1: sham-sympathectomy or sympathectomy; factor 2: control or Ang II treatment; Superanova) was used to compare SBP and vessel dimensions among the four groups of rats (Fig. 9, Table 2). For comparisons of SBP, the average weekly blood pressure of rats during the 4-week treatment

period was calculated. When the term Px for interaction between Ang II treatment and sympathectomy was not significant, the effects of the two interventions were analyzed separately. This is indicated by the variables pSx and pAng II in Table 2. When there was a significant interaction between the two interventions, statistical analysis, using preplanned contrasts (Superanova), was confined to comparisons of the effects of Ang II and control treatment between sham-sympathectomized and sympathectomized rats (Table 2). In the case of small arteries, statistical analysis of vessel dimensions was restricted to WLR because the possibility of selection bias could not be excluded, considering that measurements were performed only on cross-sectionally cut arteries. WLR of small arteries of sham-sympathectomized and sympathectomized Ang II-treated rats was correlated to their average weekly SBP by linear regression analysis. $P<0.05$ was considered statistically significant.

5.3.2. *Results*

The tail SBP of rats are shown in Fig. 9. In regard to SBP, there was a significant interaction between the effects of sympathectomy and of Ang II treatment ($P<0.02$, Table 2); without sympathectomy, Ang II treatment resulted in a significant rise in SBP ($P<0.001$), whereas the rise in SBP in sympathectomized rats during Ang II treatment did not attain statistical significance.

The morphometric measurements of first-order arteries are summarized in Table 2. Ang II and sympathectomy had independent effects on vessel morphology. Ang II treatment caused a significant increase in vessel and lumen diameters, wall thickness, and vessel, lumen, and medial wall areas. Sympathectomy, on the other hand,

resulted in minor increases in vessel dimensions but highly significant reductions in WLR and wall-to-lumen area ratio. On comparing the effects of Ang II and control treatment on sham-sympathectomized and sympathectomized rats by preplanned contrasts (Table 2), some important quantitative differences between the responses of rats of the two groups were observed. Although an increase in vessel area occured in rats of both groups during Ang II treatment, a significant increase in wall thickness and wall area occured only in sham-sympathectomized rats. The increase in wall thickness and wall area of first-order arteries in Ang II-treated sympathectomized rats did not attain statistical significance. Morphometric measurements of second-order arteries revealed the same effects of Ang II treatment and of sympathectomy as those that had been observed for the first-order arteries. Because of this, these results are not shown. The remodeling and growth indexes of first- and second-order arteries in sham-sympathectomized Ang II-treated rats were 134 and 137%, and 58 and 54%, respectively. The first set of numbers indicates that, in addition to a change in lumen diameter, there was also growth of these arteries. The second set of numbers is a measure of the extent of this growth. The remodeling and growth indexes of first- and second-order arteries in sympathectomized Ang II-treated rats were 118 and 112%, and 28 and 18%, respectively, indicating that a marked attenuation of Ang II-induced growth had occured. Finally, like in the case of SBP, Ang II treatment and sympathectomy interacted in determining the number of nuclei per vessel wall length in first-order arteries (Table 2): we observed an increase in number of nuclei only in Ang II-treated sham-sympathectomized rats.

The morphometric measurements of small- (50-100 μm outer diameter) and intermediate-size (100-150 μm outer diameter) resistance

arteries of sham-sympathectomized and sympathectomized rats are shown in Tables 2 and 3. Like in the case of first-order arteries, there was no interaction between the effects of Ang II treatment and sympathectomy in determining the WLR of resistance arteries. Compared with that in control rats, using preplanned contrasts, the increase in WLR of resistance arteries in sham-sympathectomized and sympathectomized rats was of the same magnitude. This is in sharp contrast to that which was observed in first-order (conduit) arteries, where the increases in wall thickness and wall area were limited to sham-sympathectomized rats. The WLR of intermediate-size resistance arteries in severely hypertensive sham-sympathectomized Ang II-treated rats was correlated directly to the blood pressure load to which the rats had been exposed during the 4-week treatment period ($r=0.61$, $n=11$, $P<0.05$). We found no such correlation for sympathectomized Ang II-treated rats ($r=0.09$, $n=8$, NS).

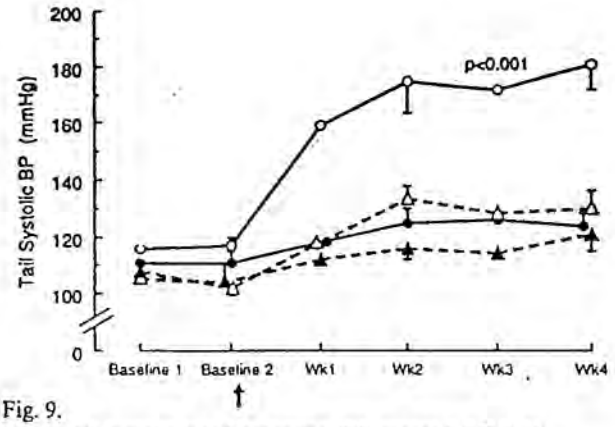

Fig. 9.

Weekly tail systolic blood pressures (BP, means ± SEM) of angiotensin II (ANG II)-treated (open symbols) and control (solid symbols) sham-sympathectomized (solid lines) and sympathectomized (dashed lines) rats. O, ANG II ($n = 11$); Δ, sympathectomized plus ANG II ($n = 10$); ●, control ($n = 8$); ▲, sympathectomized control ($n = 6$). $P < 0.001$, versus sham-sympathectomized controls (solid circles).

Table 2. Effects of sympathectomy and angiotensin II on systolic blood pressure and vessel structure determined by two-way analysis of variance (ANOVA)

	Treatment					ANOVA		
	Control		Angiotensin II		Significance	pSx	pANG II	Px
Systolic blood pressure (average of 4 weeks) (mmHg)								
Sham-SXY	124 ± 3		171 ± 8		$P < 0.001$	ND	ND	< 0.02
SXY	116 ± 9		127 ± 4		NS			
Tail artery arteries								
Vessel diameter (μm)								
Sham-SXY	331 ± 14	(n = 7)	398 ± 20	(n = 10)	$P < 0.02$	NS	< 0.01	NS
SXY	371 ± 13	(n = 6)	426 ± 18	(n = 10)	NS			
Lumen diameter (μm)								
Sham-SXY	294 ± 14	(n = 7)	349 ± 17	(n = 10)	$P < 0.05$	< 0.05	< 0.01	NS
SXY	334 ± 12	(n = 6)	385 ± 19	(n = 10)	NS			
Median wall thickness (μm)								
Sham-SXY	18.3 ± 0.7	(n = 7)	24.5 ± 1.9	(n = 10)	$P = 0.01$	NS	< 0.02	NS
SXY	18.4 ± 0.8	(n = 6)	20.7 ± 1.6	(n = 10)	NS			
WLR (%)								
Sham-SXY	6.3 ± 0.3	(n = 7)	7.1 ± 0.4	(n = 10)	NS	< 0.02	NS	NS
SXY	5.5 ± 0.2	(n = 6)	5.5 ± 0.6	(n = 10)	NS			
Vessel area (10^3 μm²)								
Sham-SXY	88 ± 8	(n = 7)	128 ± 13	(n = 10)	$P < 0.05$	NS	< 0.01	NS
SXY	108 ± 7	(n = 6)	145 ± 13	(n = 10)	$P < 0.05$			
Lumen area (10^3 μm²)								
Sham-SXY	68 ± 6	(n = 7)	96 ± 10	(n = 10)	NS	NS	< 0.01	NS
SXY	86 ± 6	(n = 6)	118 ± 11	(n = 10)	$P < 0.05$			
Wall area (10^3 μm²)								
Sham-SXY	20 ± 1	(n = 7)	32 ± 4	(n = 10)	$P = 0.01$	NS	< 0.01	NS
SXY	22 ± 1	(n = 6)	27 ± 2	(n = 10)	NS			
WALAR (%)								
Sham-SXY	30 ± 2	(n = 7)	35 ± 3	(n = 10)	NS	0.01	NS	NS
SXY	25 ± 1	(n = 6)	25 ± 2	(n = 10)	NS			
Nuclei (n/100 μm length)								
Sham-SXY	14 ± 1	(n = 7)	18 ± 1	(n = 10)	$P < 0.01$	ND	ND	< 0.01
SXY	15 ± 1	(n = 6)	14 ± 1	(n = 10)	NS			
Intermediate-size resistance arteries (100–150 μm outer diameter)								
WLR (%)								
Sham-SXY	7.0 ± 0.3	(n = 8)	8.2 ± 0.4	(n = 11)	$P < 0.02$	NS	< 0.001	NS
SXY	6.5 ± 0.2	(n = 6)	8.4 ± 0.3	(n = 8)	$P < 0.01$			
Small resistance arteries (50–100 μm outer diameter)								
WLR (%)								
Sham-SXY	8.7 ± 0.3	(n = 7)	11.4 ± 0.6	(n = 8)	$P < 0.02$	NS	< 0.01	NS
SXY	8.9 ± 0.5	(n = 6)	11.4 ± 1.1	(n = 6)	$P < 0.05$			

Values are means ± SEM. pSX, sympathectomy alone; pANG II, angiotensin II alone; Px, interaction term; SXY, sympathectomy; ND, not done; WLR, wall : lumen ratio; WALAR, medial wall : lumen area ratio.

Table 3. Morphometry of small and intermediate-size mesenteric resistance arteries of angiotensin II (ANG II)-treated sympathectomized and sham-sympathectomized rats and of control rats

Variable	Sham-sympathectomized				Sympathectomized			
	Control		ANG II-treated		Control		ANG II-treated	
Small resistance arteries (50–100 μm outside diameter)								
Vessel diameter (μm)	81 ± 2	(n = 7)	78 ± 2	(n = 8)	80 ± 2	(n = 6)	79 ± 4	(n = 6)
Lumen diameter (μm)	69 ± 1	(n = 7)	63 ± 2	(n = 8)	68 ± 2	(n = 6)	65 ± 3	(n = 6)
Medial wall thickness (μm)	5.9 ± 0.2	(n = 7)	7.1 ± 0.3	(n = 8)	5.9 ± 0.2	(n = 6)	7.2 ± 0.5	(n = 6)
WLR (%)	8.7 ± 0.3	(n = 7)	11.4 ± 0.6	(n = 8)	8.9 ± 0.5	(n = 6)	11.4 ± 1.1	(n = 6)
Intermediate-size resistance arteries (100–150 μm outside diameter)								
Vessel diameter (μm)	114 ± 2	n = 8)	132 ± 5	(n = 11)	118 ± 2	(n = 6)	135 ± 5	(n = 8)
Lumen diameter (μm)	101 ± 2	(n = 8)	113 ± 4	(n = 11)	104 ± 2	(n = 6)	116 ± 4	(n = 8)
Medial wall thickness (μm)	7.0 ± 0.1	(n = 8)	9.3 ± 0.5	(n = 11)	6.8 ± 0.3	(n = 6)	9.8 ± 0.6	(n = 8)
WLR (%)	7.0 ± 0.3	(n = 8)	8.2 ± 0.4	(n = 11)	6.5 ± 0.2	(n = 6)	8.4 ± 0.3	(n = 8)

Values are means ± SEM. For statistical analysis of wall : lumen ratios (WLR).

5.3.3. *Summary*

Hypertension and sympathetic innervation appear to be contributing to the development of structural changes in large arteries of Ang II-treated rats because sympathectomy attenuated these changes. Structural changes in small arteries, on the other hand, appear to be due to a direct trophic effect of Ang II.

5.4. *Structural vascular changes in hypertension – role of Ang II, dietary sodium supplementation, blood pressure and time*

5.4.1. *Methods*

Design of experiments. Pathogen-free male Sprague-Dawley rats were used throughout these experiments. Ang II was administered to rats in three different doses (50, 100 and 200 $ng \cdot kg^{-1} \cdot min^{-1}$) for 4 to 12 weeks to test the hypothesis that the agonist exerts a dose- and time-dependent effect on the development of hypertension and of structural vascular changes. Additional rats received high-sodium (2% NaCl) diet alone or in combination with 50 $ng \cdot kg^{-1} \cdot min^{-1}$ Ang II for 12 weeks to test for a synergistic effect of the two stimuli. It has been estimated that 2% NaCl diet for rats corresponds to daily intake of ≈15 g of salt by humans (59), a salt load that sometimes is encountered in clinical practice. Sham-operated rats on normal sodium diet for 4-12 weeks served as controls for all the treatment groups, including the rats on high-sodium diet.

The *preparation of rats* and *blood pressure measurements* have been described in details in the previous chapters (Chapter 5.1.1., 5.2.1. and 5.3.1.).

Intra-arterial blood pressure measurements. For direct measurement of BP, rats were fitted with a femoral artery catheter 7-10 days before the end of the 4- or 12-week treatment period. The operation was performed in anesthetized rats. A polyvinyl chloride catheter was advanced into the abdominal aorta through the femoral artery. The distal end of the catheter was tunneled subcutaneously from the groin to the back of the neck, where it was exteriorized. After a 3-day recovery period, the arterial catheter was connected to a pressure transducer (Kent Scientific Corp.), and the mean arterial BP (MAP) was monitored continuously for 2 hours between 8 AM and 12 PM on 3 separate days. During monitoring, rats moved freely in their boxes. The filtered analog signals were digitalized and sampled using a computer. The average MAP was calculated from collected data for each day, and the average daily MAP during 3 days of measurement was calculated.

Plasma Ang II concentration measurements. At the completion of direct BP measurements, arterial blood samples were obtained from 7 awake rats receiving 100 $ng \cdot kg^{-1} \cdot min^{-1}$ Ang II sc and from 15 control rats. Plasma Ang II concentrations (pg/mL) were measured using high-pressure liquid chromatography fractionation and radioimmunoassay (60).

In situ tissue fixation and *morphometric measurements* have been described in details in the previous chapters (Chapter 5.1.1., 5.2.1. and 5.3.1.). In the present study, two categories of resistance arteries, one with external diameter of 50 to 100 μm (small) and the other with external diameter of 100 to 150 μm (intermediate-size) were investigated. Measurements were made on a minimum of 3 cross-sectionally cut arteries in each of the two vessel categories investigated. Wall thickness and WLR and the mean of each parameter were calculated for the two categories of vessels for each rat. For further

characterization of structural vascular changes induced by Ang II treatment, four 2nd-order mesenteric arteries (250 to 350 μm OD) were removed from each of nine rats treated with 100 ng·kg^{-1}·min^{-1} Ang II for 12 weeks and from seven 12-week control rats. The arteries were dehydrated through graded series of ethanol and embedded in epoxy resin. Cross sections of 1 μm thickness were stained with toluidine blue. A video camera connected to an image analysis processor and microcomputer was used to measure lumen diameter, wall thickness, the number of smooth muscle cell layers, and cell thickness. The latter three parameters were measured at 4 points where arbitrarily drawn horizontal and vertical axes intersected the vessel wall. Cell thickness was measured at the midpoint of the nucleus along the short axis of the smooth muscle cell. Measurements obtained from 2 to 4 arteries per rat were averaged. WLR was calculated.

Statistical analysis. Repeated measures ANOVA (1 factor within, 1 between) was used to compare weekly BPs of Ang II-treated rats and of sodium-supplemented rats with those of control rats. Linear regression analysis was used to correlate the average weekly tail SBP and the average MAP of catheterized rats.

Statistical analysis of small and intermediate-size artery dimensions was restricted to WLRs. WLRs in the 7 groups of variously treated rats were compared with those of control rats by 2-factor ANOVA (factor 1, treatment; factor 2, small- and intermediate-size arteries) followed by preplanned contrasts. To compare the dimensions of large arteries of rats treated with 100 ng·kg^{-1}·min^{-1} Ang II for 12 weeks and of matched control rats, the 2-factor ANOVA was extended to include the large arteries. In Ang II-treated rats, the contribution of dose and duration of treatment to changes in WLR was tested with 2-factor ANOVA (factor 1, dose; factor 2, 4 or 12 weeks of treatment).

For this analysis, only rats on a normal sodium diet (0.7% NaCl) receiving 50, 100, or 200 $ng \cdot kg^{-1} \cdot min^{-1}$ Ang II for 4 or 12 weeks were included. One-way ANOVA was used to compare plasma Ang II concentrations in rats receiving 100 $ng \cdot kg^{-1} \cdot min^{-1}$ Ang II sc for 4 weeks with those of control rats. Null hypotheses were rejected at $P<0.05$.

5.4.2. *Results*

The tail SBP of rats is shown in Figures 10 and 11. There was a rapid development of severe hypertension in rats receiving 200 $ng \cdot kg^{-1} \cdot min^{-1}$ Ang II sc for 4 weeks. The development of hypertension was more gradual in rats treated with the 100 $ng \cdot kg^{-1} \cdot min^{-1}$ dose of Ang II, reaching moderate levels by ≈4 weeks of treatment. In rats treated with 50 $ng \cdot kg^{-1} \cdot min^{-1}$ Ang II, BP rose transiently at 3 and 4 weeks but returned to baseline by the end of the 12-week treatment period (Figures 10 and 11). During the 12 week treatment, the BP of rats treated with 50 $ng \cdot kg^{-1} \cdot min^{-1}$ Ang II was not different from that of control rats. The BP curve of control rats and of rats receiving the 2% NaCl diet overlapped during the entire treatment period. However, when the 2% NaCl diet was combined with the administration of 50 $ng \cdot kg^{-1} \cdot min^{-1}$ Ang II, SBP of rats began to rise by week 3, reaching mildly elevated levels by week 12. At the end of 4 weeks of treatment, the plasma Ang II level of rats receiving 100 $ng \cdot kg^{-1} \cdot min^{-1}$ Ang II sc (26±5 pg/mL, n=7) was increased compared with that of control rats (11±2 pg/mL, n=15, $P<0.03$).

The WLRs of small and intermediate-size mesenteric resistance arteries of rats in the various treatment groups are summarized in Figures 12 and 13. A dose-dependent increase in the WLR of both categories of vessels was observed, but the increases were generally greater in small

arteries. The increase in the WLR of small arteries of rats treated with 50 $ng \cdot kg^{-1} \cdot min^{-1}$ Ang II for 12 weeks was of borderline statistical significance ($P<0.06$, Figure 12). The effect of dose and time of administration of Ang II on WLR was analyzed by 2-factor ANOVA. In the small arteries, there was a significant independent effect of dose of Ang II ($P<0.01$) but not of duration of treatment ($P<0.20$) on wall thickening (Figure 12). In intermediate-size arteries, the effect of both dose and duration of treatment was statistically significant ($P<0.02$ and $P<0.01$, respectively, Figure 4).

Like the increase in WLR of small arteries of rats treated with 50 $ng \cdot kg^{-1} \cdot min^{-1}$ Ang II for 12 weeks, the increase in WLR of small arteries of rats treated with 2% NaCl diet for 12 weeks was of borderline statistical significance ($P<0.07$). Interestingly, 9 of the 17 rats displayed an increase in WLR (10.7%, mean), whereas the remaining 8 rats had no response at all (WLR=8.5%, mean). When the 2% NaCl diet of rats was combined with the administration of 50 $ng \cdot kg^{-1} \cdot min^{-1}$ Ang II for 12 weeks, a highly significant increase in WLR was achieved (synergism).

WLR of both categories of rats was increased in Ang II-treated rats. Statistical comparison of large-artery dimensions in the two groups revealed increased wall thickness, WLR, and vascular smooth muscle cell thickness in Ang II-treated rats without a change in the number of smooth muscle cell layers. This finding indicates vascular muscle hypertrophy rather than hyperplasia.

A high degree of correlation was found between directly measured MAP and the average weekly tail SBP of rats indicating that the latter was an acceptable measure of the BP load of rats. This permitted us to construct the final summary figure (Figure 14) on which the WLRs of small arteries of the various treatment groups are plotted against their average BP load, calculated as the area under the SBP curves of rats

(Figures 10 and 11). Included in this figure are data from the previous study in which neonatally sympathectomized rats were treated with 200 $ng \cdot kg^{-1} \cdot min^{-1}$ Ang II sc for 4 weeks or sham treatment. The figure illustrates that there is a dissociation between the BP load that these rats were exposed to and the WLR of their small mesenteric arteries, especially in the earliest stages of hypertension. This impression was confirmed by linear regression analysis of the BP load and WLR of small mesenteric arteries of individual rats that were treated with Ang II for 4 weeks ($r=0.15$, $n=32$, NS) or for 12 weeks ($r=0.04$, $n=23$). A similar dissociation was found between the BP load of rats and the WLR of their intermediate-size mesenteric arteries.

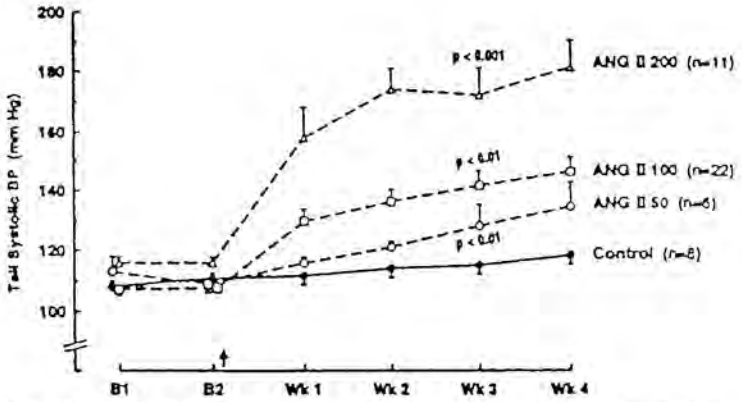

Fig. 10. Tail SBP (mean±SEM) of rats treated with 50, 100, or 200 ng · kg^{-1} · min^{-1} Ang II SC for 4 weeks and of control rats. The BP curve of Ang II–treated rats was compared with that of control rats by repeated-measures ANOVA. B1 and B2 indicate 1st and 2nd baseline BP; arrowhead indicates start of treatments.

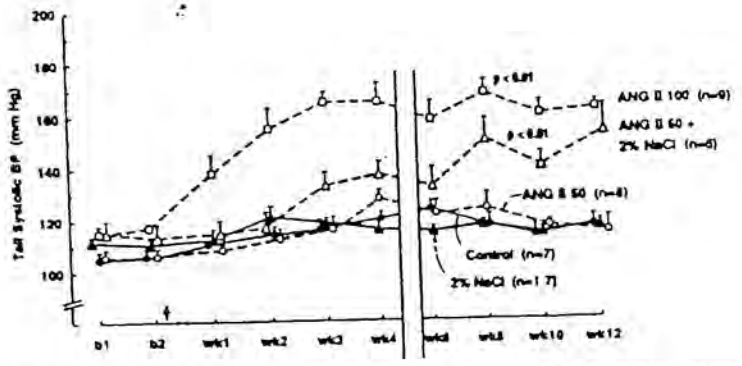

Fig. 11. Tail SBP (mean±SEM) of rats treated with 50 or 100 ng · kg^{-1} · min^{-1} Ang II SC, 2% NaCl diet, or 2% NaCl diet+50 ng · kg^{-1} · min^{-1} Ang II SC for 12 weeks and of control rats. The BP curve of treated rats, including the rats on 2% NaCl diet, was compared with that of control rats by repeated-measures ANOVA.

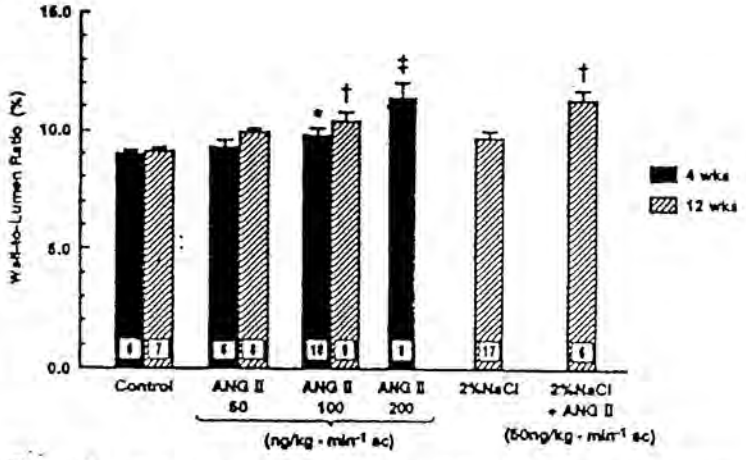

Fig. 12.. Wall-to-lumen ratio (mean±SEM) of small mesenteric resistance arteries (50 to 100 μm OD) of rats on the various 4-week (solid bars) and 12-week (hatched bars) treatment regimens; number of rats is shown at the base of each bar. W/L of Ang II–treated and 2% NaCl-fed rats was compared with that of appropriate control rats by 2-factor ANOVA. *$P<0.05$; †$P<0.01$; ‡$P<0.001$.

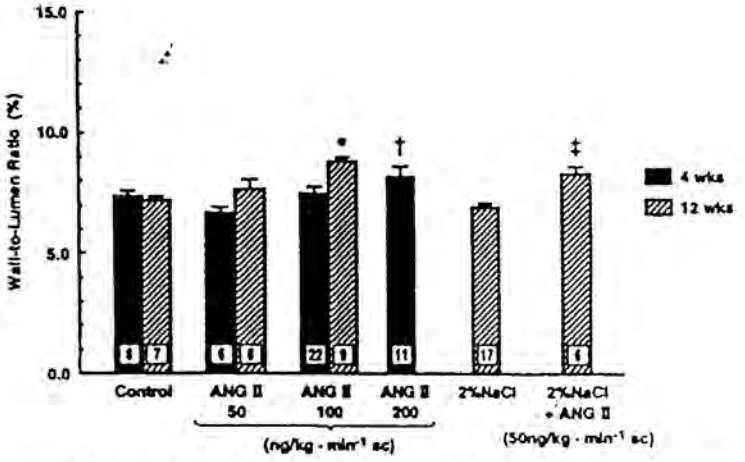

Fig. 13. Wall-to-lumen ratio (mean±SEM) of intermediate-size mesenteric resistance arteries (100 to 150 μm OD) of rats on the various 4-week (solid bars) and 12-week (hatched bars) treatment regimens; number of rats is shown at the base of each bar.
*$P<0.01$; †$P<0.05$; ‡$P<0.02$.

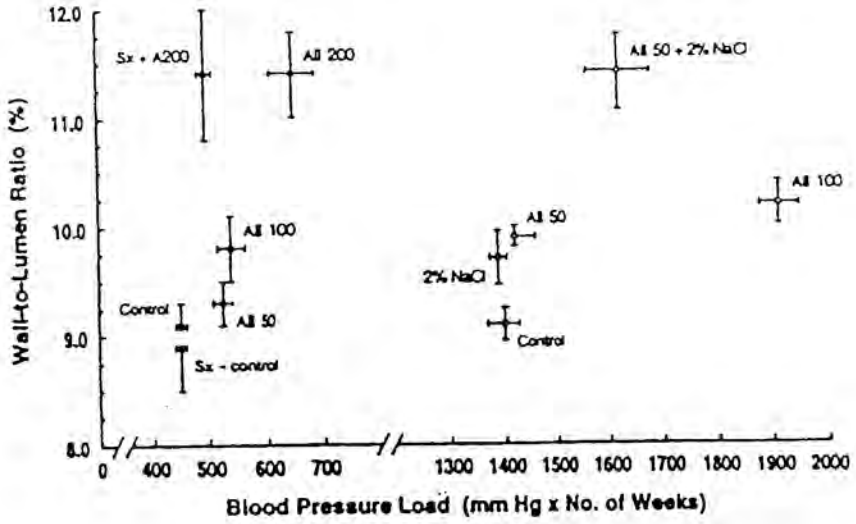

Fig. 14. Graphic plot of the average wall-to-lumen ratio (mean±SEM) of small mesenteric resistance arteries (50 to 100 μm OD) of the various treated groups of rats against their BP load (the area under the weekly SBP curve of rats) (mean±SEM) compared with that of control rats. ● and ○ indicate 4- and 12-week treatment, respectively; 50, 100, and 200 indicate the dose of Ang II in ng · kg^{-1} · min^{-1} SC. Included are sympathectomized control rats (Sx) and sympathectomized rats treated with 200 ng · kg^{-1} · min^{-1} Ang II SC (Sx+A II 200) from a previous study.[17]

5.4.3. *Summary*

Ang II-induced hypertension and structural vascular changes are dose- and time-dependent and synergistically enhanced by dietary sodium supplementation. Dissociation between BP and vascular structure in Ang II-treated rats suggests that a direct trophic effect of Ang II may contribute to the development of structural vascular changes.

5.5. *Hypertension and progression of the kidney disease in patients with IgA nephropathy*

5.5.1. *Patients and methods*

One hundred twenty-six non-uremic patients with IgA nephropathy confirmed by renal biopsy were selected consecutively. Fifty-five patients (36 male, 19 female) were normotensive; age: 37.7±10 years (mean±SD). Normotension and hypertension was defined according to the WHO criteria. Seventy-one patients (57 male, 14 female) were hypertensive; age: 46.4±12 years (mean±SD). ABPM was carried out using an automatic device (Meditech ABPM-02, Meditech Kft., Hungary) based on the cuff-oscillometric method. ABPM readings were taken on the patients nondominant arm every 15 minutes around the clock day-time (from 6.00 am to 22.00 pm) and every 20 minutes night-time (from 22.00 pm to 6.00 am). Office blood pressures are expressed as mean values of at least three casual measurements taken at three different visits in sitting position in standardized fashion using appropriately sized cuffs and a random-zero mercury sphygmomanometer on the nondominant arm. After the ABPM the patiens were followed prospectively.

Hypertensive patients (n=71) were mild or borderline hypertensives according to the WHO criteria, treated with ACEI in monotherapy (n=43) or in combination with CCB agents (n=28).

The cutoff points for clinic and ambulatory pressure used to define „white coat hypertension" were the following: clinic blood pressure over 140/90 mmHg; 24-hour mean ambulatory blood pressure less than 135/85 mmHg (48). Clinic blood pressures considered were averages of at least three readings taken at different visits according to the WHO criteria.

„Dippers" were classified as patients having mean night-time systolic blood pressure reduction of at least 10% compared to the corresponding day-time values; "non-dippers" were classified as those, whose night-time blood pressure did not drop or was not reduced by at least 10% (34, 52, 67).

Ninety-five patients have been followed up for 36±4.1 months. At the time of the ABPM and at the end of the follow-up period, serum creatinine levels of the different groups („dipper" and „non-dipper" normotensive, „white coat hypertensive" and real hypertensive patients) were measured and compared.

Variables of interest were described in terms of their means and standard deviation. Statistical evaluations were performed by Student's t test.

5.5.2. *Results*

Diurnal blood pressure variation. Normotensive IgA nephropathy patients (n=55) had significantly higher day-time than night-time systolic and diastolic blood pressure. In treated hypertensive IgA nephropathy patients (n=71) the mean day-time systolic and diastolic blood pressure

did not differ from the mean night-time blood pressures (Figure 15). Eighty-two per cent of the normotensive patients were „dippers". Ninety-three per cent of the hypertensives were „non-dippers".

"White-coat hypertension". Ten normotensive patients were classified as hypertensives on the basis of office blood pressure measurements (149±7/96±8 mmHg) and had significantly lower, normal 24-hour mean blood pressure measured with ABPM (127±6/83±5 mmHg, $p<0.05$, Table 4). We have observed the „white-coat effect" in 14 treated hypertensives, too (mean office blood pressure 152±8/98±6 mmHg, mean 24-hour blood pressure 130±4/85±8 mmHg, $p<0.05$, Table 4).

Effectiveness of antihypertensive therapy. The mean day-time blood pressure of normotensive and treated hypertensive IgA nephropathy patients was not different. Normotensives had significantly lower night-time and 24-hour blood pressure, than treated hypertensives (Figure 15).

Progression and blood pressure. The serum creatinine of 43 normotensive (age: 38.6±10 years) and 52 hypertensive patients (age: 47.2±12 years) has been compared at the time of the ABPM and 36±4.1 months following the ABPM. Thirty-six months after the ABPM treated hypertensives had significantly higher serum creatinine than at the time of the ABPM ($p<0.05$). The serum creatinine of normotensive patients was not higher at the end of the follow-up period than at the time of the ABPM (Table 5). There was no difference in mean body weight and male/female ratio between the groups. Thirty-six ± 4.1 months after the ABPM, the serum creatinine of "dipper" normotensives was not higher than at the time of the ABPM. The serum creatinine of "non-dipper" normotensives was significantly higher at the end of the follow-up period than at the time of the ABPM ($p<0.05$, Table 5). There was no

difference in mean body weight and male/female ratio between „dippers" and „non-dippers".

The mean serum creatinine of „white-coat hypertensives" (n=10) was not different from that of other („real") normotensives (n=33) at the beginning of the 36 months follow-up period, but it was significantly higher at the end of it. The serum creatinine of „real" normotensive patients did not change during the follow-up period (Table 5).

Table 4. 'White-coat effect' in normotensive and treated hypertensive IgANP patients ($n=24$)

	n	Office blood pressure (mmHg, mean ± SD)	P	Mean 24-h blood pressure (ABPM) (mmHg, mean ± SD)
Normotensive ($n=55$)	10 (18%)	149 ± 7/96 ± 8	$P<0.05$	127 ± 6/83 ± 5
Hypertensive ($n=71$)	14 (20%)	152 ± 8/98 ± 6	$P<0.05$	130 ± 4/85 ± 8

Table 5. Serum creatinine of IgA nephropathy patients at the beginning and at the end of the follow-up period

	Serum creatinine at the time of the ABPM (μmol/l, mean ± SD)	P	Serum creatinine 36 ± 4.1 month after the ABPM (μmol/l, mean ± SD)
Normotensives ($n=43$)	89 ± 17	n.s.	93 ± 22
Hypertensives ($n=52$)	101 ± 28	$P<0.05$	124 ± 32
'Dipper' normotensives ($n=28$)	81 ± 14	n.s.	84 ± 18
'Non-dipper' normotensives ($n=8$)	89 ± 18	$P<0.05$	106 ± 17
'Real' normotensives ($n=33$)	90 ± 16	n.s.	89 ± 22
'White coat hypertensives' ($n=10$)	88 ± 19	$P<0.05$	105 ± 19

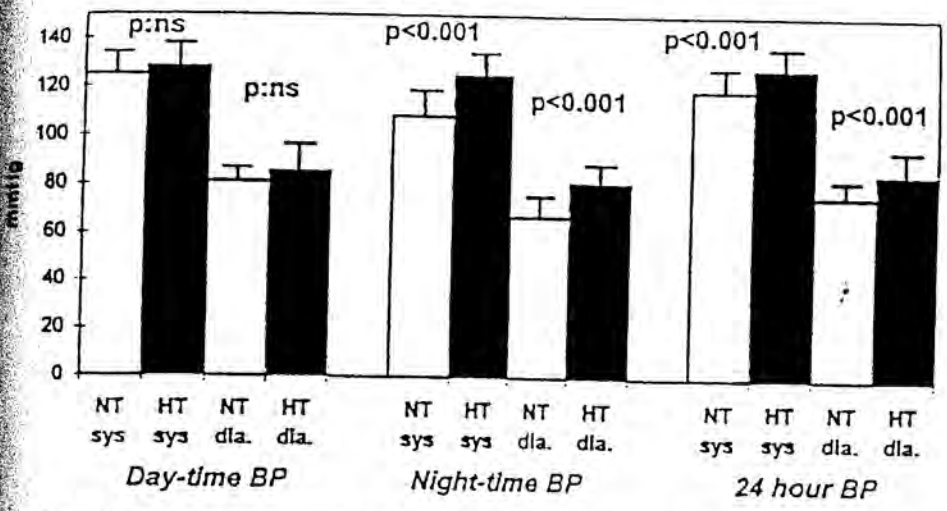

Fig. 15. Blood pressure of normotensive and hypertensive IgANP patients ($n = 126$). NT, normotensive patients; HT, treated hypertensive patients; sys, systolic blood pressure; dia, diastolic blood pressure.

5.5.3. *Summary*

There is no diurnal blood pressure variation in most of the hypertensive IgA nephropathy patients. ACEI treatment alone or in combination with CCB treatment has better effect on day-time than night-time hypertension. The lack of the circadian rhythm and „white-coat hypertension" seems to accelerate the progression of IgA nephropathy.

6. DISCUSSION

Elevated circulating or tissue levels of Ang II seem to play a pathogenetic role in almost every form of human hypertension. Different effects of Ang II, dietary sodium supplementation and sympathectomy have been studied on the development of vascular hyperresponsiveness, hypertension and structural vascular changes in a well established animal model of „high-renin" hypertension. In a clinical study, in IgA nephropathy, a typical form of human secondary hypertension, the 24-hour blood pressure profile, „white-coat hypertension", the effect of medication (ACEI in monotherapy or in combination) on the 24-hour blood pressure parameters, and the role of the above factors in the progression of the kidney disease have been investigated.

6.1. *The effect of dietary sodium supplementation on Ang II-induced hypertension*

Salt and Ang II (renin) have been recurrent themes of clinical hypertension and of hypertension research for the past 100 years. The administration of Ang II and of dietary sodium supplementation in small doses but over long periods of time promises to be a fruitful experimental approach toward understanding human essential hypertension.

The findings of the present study indicate that the interaction between dietary sodium and Ang II may be specific to this agonist and is occuring on the vascular level. For short-term administration, the mechanism whereby these two stimuli interact appears to be facilitation of sympathetic neurotransmission. Long-term dietary sodium

supplementation may potentiate the trophic vascular effects of Ang II and thereby accelerate the onset of hypertension.

Short-term mesenteric vascular responses. Reports of a direct vascular effect of dietary sodium supplementation date back many years (15, 16, 17). Long-term moderate sodium supplementation, either 2% NaCl diet or 1-2% saline for drinking water, increased reactivity of the aorta and tail artery of rats, measured in vitro, to epinephrine, NE and Ang II, but the rats remained normotensive. To produce hypertension, rats were treated with 1% saline for drinking water and 1% NaCl diet for up to 1 year (61). After 5-8 months of treatment, lesions of necrotizing arteritis were observed in some of the rats. Because of the large amount of obligatory sodium intake to which these rats were exposed, the relevance of these experiments to human hypertension is difficult to assess. High-sodium diet increases the number of renal α2-adrenergic receptors and α-adrenergic responsiveness of the aorta of rats (16, 62), but it is not known whether potentiation of vascular responses to catecholamines is due to postsynaptic or presynaptic mechanisms.

In our study, dietary sodium supplementation by itself had no effect on the BP of rats but potentiated vasoconstrictor responses to Ang II and nerve stimulation. The fact that vasoconstrictor responses to NE and AVP in the same rats were unchanged argues against the role of structural vascular changes or of circulating vasoconstrictor substances in the pathogenesis of increased vascular reactivity. Although increased vasoconstrictor responses to Ang II may be explained by upregulation of its receptors as a result of supression of circulating Ang II levels by high-sodium diet (63), hyperreactivity to nerve stimulation suggests that additional mechanisms are also involved. Facilitation of sympathetic neurotransmission in the present study appears to be presynaptic, because vasoconstrictor responses to NE in the same preparations were

unchanged. The immediate effect of the central nervous system on sympathetic activity was abolished by severing the nerves surrounding the SMA of rats.

These effects of dietary sodium supplementation resemble the effects of Ang II administration to rats (10, 11, 12, 64). The administration of Ang II in initially subpressor doses results in a shift of the Ang II pressure-dose response curve toward the pressure axis, a phenomenon that was termed „autopotentiation" (10, 11). In rats treated with subpressor doses of Ang II, it has been shown that autopotentiation of pressor responses was due to increased vasoconstrictor responses in the mesentery and was accompanied by facilitation of sympathetic neurotransmission during peripheral nerve stimulation (12, 64). Structural vascular changes were not found at this stage of the hypertensive process (13).

When Ang II treatment and dietary sodium supplementation of rats were combined, vasoconstrictor responses to Ang II were greatly exaggerated, again in an agonist-specific manner. It is logical to suggest that the basis of this synergism is the summation of the individual effects of the two treatments. The hypothesis that extracellular sodium content is a determinant of the transmembrane sodium gradient, which in turn determines the magnitude of the vasoconstrictor responses to agonists whose mode of action includes the stimulation of sodium influx (64), has been advanced earlier. Ang II is such an agonist. By stimulating the synthesis of cation-binding glycosaminoglycanes, Ang II may increase the transmembrane sodium gradient and, thereby, potentiate its own vasoconstriction (66). One would expect that dietary sodium supplementation would augment this response by increasing the transmembrane sodium gradient further.

Transmembrane sodium gradient may also determine the transport and storage of NE in sympathetic nerve endings, an increase in gradient favoring increased uptake and storage of the neurotransmitter (19, 20). Thus the postulated increase in transmembrane sodium gradient may explain both the increased vasoconstrictor response to Ang II and facilitation of sympathetic neurotransmission during nerve stimulation in sodium-fed rats. Finally, although high-sodium diet and Ang II treatment acted synergistically in increasing vasoconstrictor responses to Ang II, no such interaction occured during periarterial nerve stimulation. It appears that maximal facilitation of neurotransmission already occured with either Ang II treatment or high-sodium diet.

In contrast to Ang II treatment and dietary sodium supplementation, PE treatment alone or in combination with high-sodium diet had no hemodynamic effects. This lack of response to PE did not seem to be secondary to downregulation of α-adrenergic receptors, because responses to NE and nerve stimulation were unchanged in PE-treated rats.

Long-term pressor and structural vascular effects. In the mesenteric circulation of rats, resistance arteries are the 4th and 5th order branches of the SMA and have an external diameter less than 150 μm. These are the arteries whose WLR was measured in the present study. In rats treated with 50 ng·kg^{-1}·min^{-1} Ang II sc for 12 weeks, there was no significant elevation of SBP compared with that of control rats, yet the WLR of small resistance arteries (<100 μm OD) was increased. The WLR of intermediate size arteries was unchanged. Interestingly, the WLR of small arteries was also increased in rats fed a high-sodium diet. The response was, however, heterogenous; some rats had a clear increase in the WLR of small arteries while others had no response at all. These findings suggest that some of these Sprague-Dawley rats may be salt

sensitive while others are not. If confirmed, this finding would represent a new definition of salt sensitivity, i.e., as a trophic vascular response that may precede the onset of hypertension. The development of structural vascular changes may explain the nonselective increase in vascular reactivity that previous investigators have reported in rats maintained on a high-sodium diet for several months (15, 17, 61). The synergistic pressor action of long-term Ang II treatment and of dietary sodium supplementation in the present study may be explained by the additive trophic vascular effect of the two stimuli.

Unlike Ang II, PE either alone or in combination with dietary sodium supplementation had no effect on either the BP or WLR of resistance arteries of rats. An important difference in the long-term effects of Ang II and PE has emerged: Ang II is a slow pressor agent, especially in combination with high-sodium diet, and a trophic factor of vascular muscle, while PE is neither.

We speculate that the short-term vasoconstrictor effects of Ang II treatment and of dietary sodium supplementation are causally linked to their long-term pressor and trophic vascular effects. It has been proposed that it is vasoconstrictor hyperresponsiveness and the associated increases in pressor responses that initiate the positive feedback that leads to the development of structural vascular changes and the establishment of hypertension (28, 32, 68). Our findings support this proposed sequence of events. By potentiating the vasoconstrictor and trophic vascular responses to Ang II, high-sodium diet serves as a „reinforcement" of the hypertensive stimulus (28, 32, 69). The findings also help to localize the segment of the arterial circulation where structural vascular changes are initiated and indicate that these changes may occur before the onset of hypertension.

6.2. *Effect of neonatal sympathectomy on development of Ang II-induced hypertension*

There are two ways to investigate the role of the sympathetic nervous sytem in the development of Ang II-induced hypertension. One is to measure sympathetic activity during chronic Ang II excess, and the other is to test whether the long-term pressor or vasoconstrictor action of Ang II can be inhibited by blocking or destroying the sympathetic nervous system. Infusion of Ang II into the vertebral artery of dogs for 7 days resulted in hypertension that could be abolished by the administration of bretylium or by cervical section of the spinal cord (70, 71, 72). The site of action of Ang II appears to be the area postrema in the medulla oblongata, where fenestration in the blood brain barrier allows direct access to the central nervous system. These findings were confirmed in part in dogs and rats receiving systemic infusion of pressor doses of Ang II (73, 74).

The experiments aimed at preventing Ang II-induced hypertension by inhibiting or destroying sympathetic pathways have been generally unsuccessful, with one exception, that of surgical ablation of the area postrema in the medulla oblongata. Although surgical ablation of the area postrema attenuated the slow pressor effect of Ang II (75, 76), guanethidine infusion in dogs and renal denervation or 6-hydroxydopamine treatment of rats failed to prevent or attenuate the hypertension induced by pressor doses of Ang II (13, 25, 26). When Ang II is administered in pressor doses, the salt and water retention that results from the direct vasoconstrictor action of the agonist and the rapid rise of BP may mask or negate all other potential pressor mechanisms, including sympathetic hyperactivity (10, 11, 75). In contrast, when Ang II is administered in small or initially subpressor doses as in the present

study, the development of hypertension is gradual, and salt and water retention is undetectable (10, 11). It is in this setting that the interaction between the renin-angiotensin and sympathetic nervous system may be detected.

To achieve sympathectomy, we used the technique developed by Johnson et al. (57). They documented that the administration of guanethidine to newborn rats combined with bilateral adrenal medullectomy produces a virtually complete and permanent sympathectomy. Plasma NE and epinephrine levels in these rats are reduced 94% and 96%, respectively, compared with those of sham-injected and sham-operated controls (77). As expected, sympathectomized rats displayed vasoconstrictor hypersensitivity to NE and markedly reduced to absent vasoconstrictor responses to periarterial nerve stimulation. Vasoconstrictor responses to Ang II and AVP of sympathectomized rats were unchanged compared to those of sham-sympathectomized rats.

Sympathectomy prevented autopotentiation of vasoconstrictor responses in the early stages of Ang II administration and the generalized increase in mesenteric vasoactivity during chronic Ang II administration. Because these measurements were carried out in an isolated regional vascular bed pump-perfused at constant flow, these changes must be ascribed to direct vascular effects of sympathectomy rather than to nonspecific systemic effects. In contrast to the hemodynamic responses to Ang II, sympathectomy had no effect on Ang II-induced structural vascular changes.

There is evidence that the sympathetic nervous system controls many of the processes involved in the excitation-contraction coupling of vascular smooth muscle, including interaction of agonists with receptors, depolarization of cell membrane, increase in cytoplasmatic free calcium

from extracellular and intracellular stores, and phosphorylation and interaction of free cellular calcium with contractile protein (78). Sympathectomy, therefore, may alter the contractile response and thus the sensitivity of vascular muscle by interfering with some or all of these processes. The interesting finding of our study is that sympathectomy by itself did not sem to affect the vasoconstrictor function of sham-treated (control) rats, as evidenced by unaltered responses to Ang II and AVP. Sympathectomy affected mainly the response to Ang II treatment, raising the possibility of an interaction between the renin-angiotensin system and the sympathetic nervous system on the vascular level.

In the early stage of Ang II administration, sympathectomy prevented the autopotentiation of vasoconstrictor responses by Ang II. In the abscence of autopotentiation, pressor hyperresponsiveness that is required to set in motion the vicious circle that leads to hypertension may be interrupted, thus explaining the absence of chronic hypertension in our sympathectomized, Ang II-treated rats (28).

In Ang II-treated rats, sympathectomy did not prevent the development of structural vascular changes. This has been an unexpected finding, considering that hypertension to a large extent was prevented. It has been generally accepted that the presence of hypertension correlates best with the presence of structural vascular changes, and an increase in WLR is the predominant lesion (27, 28). In the majority of studies of hypertension, there has been a direct relationship between BP and elevation of WLR (28, 30, 31, 78). In the present study, on the other hand, we found a virtual absence of hypertension and unchanged vasoconstrictor responses to agonists in the presence of structural vascular changes, that is, a dissociation between function and structure. From this, we must conclude that the vasoconstrictor responses to agonists of sympathectomized Ang II-treated rats are subnormal. This

abnormality in vasoconstrictor responses may explain the absence of hypertension in the presence of structural vascular change. Taken together, the findings indicate that structural vascular changes by themselves are not sufficint to produce hypertension and suggest that normally functioning vascular muscle is also needed to maintain the vasoconstrictor tone of restructured resistance arteries. Simply put, to produce hypertension both structural vascular changes and increased vascular reactivity or vasoconstrictor input are needed.

The development of structural vascular changes in sympathectomized, Ang II-treated rats in the virtual absence of hypertension provides in vivo evidence for the trophic effects of Ang II. In vivo evidence for the trophic vascular effect of Ang II has been less extensive than in vitro evidence, using vascular smooth muscle cells in tissue culture (80). In familial chronic diarrhea and in Bartter's syndrome (clinical conditions of high plasma renin activity), vascular hypertrophy develops in the absence of hypertension (81, 82). In rats treated with pressor doses of Ang II, antihypertensive therapy prevented the hypertension but not the development of vascular hypertrophy (33). It has been provided evidence for stimulation of the vascular Na-K pump, a requirement for growth, and increased protein and glycosaminoglycan synthesis of vascular muscle in rats receiving subpressor doses of Ang II (66, 83, 84). The findings of the present study extend these in vitro observations. The increase in WLR of resistance arteries of sympathectomized rats treated with Ang II was as great as that of sham-sympathectomized Ang II-treated rats, despite the fact that the rise of BP in the former was minimal or nonexistent. In the absence of sympathetic innervation and of hypertension, the direct trophic effect of Ang II appears to be responsible for the structural vascular changes.

In summary, neonatal sympathectomy prevented autopotentiation of vasoconstrictor responses in the early stages of Ang II administration and the generalized increase in mesenteric vasoactivity during chronic Ang II administration. The functional consequence of this was the absence of chronic hypertension. Sympathectomy did not prevent the structural vascular changes induced by chronic Ang II administration, supporting a direct trophic vascular effect of the agonist. Structural vascular changes by themselves appear to be insufficient to produce hypertension. Increased vascular reactivity or vasoconstrictor input is also required.

6.3. *Effect of neonatal sympathectomy on the development of structural vascular changes in Ang II-treated rats*

Morphometric measurements of mesenteric arteries in rat models of hypertension have been performed before using myographic and morphometric techniques (27, 85). For the most part, use of myographic techniques has been confined to vessels with external diameters greater than 250 μm (27, 33). Lee et al. (85) developed techniques that permitted morphometric measurements on mesenteric arteries with external diameters in the 120-150 μm range, slightly larger than the intermediate-size arteries investigated in the present study. The techniques used by us also permitted measurements to be performed on arteries with external diameters in the 50-100 μm range, some of which may be considered arterioles.

Sympathectomy alone resulted in an increase in dimensions and decrease in WLR and wall-to-lumen area ratio of first- and second-order branches of the superior mesenteric artery (large arteries). An increase in vessel lumen accompanied by a decrease in WLR was termed

hypotrophic outward remodeling (86). A trophic effect of sympathectomy on vessel dimensions had previously been reported (87). In our study, neonatal sympathectomy had no significant effect on WLR of small mesenteric resistance arteries.

There were important differences between the responses of small and large arteries to Ang II treatment and in the effect of neonatal sympathectomy on these responses. With the development of severe hypertension in Ang II-treated sham-sympathectomized (intact) rats, the large arteries underwent a marked increase in medial wall area and dilated at the same time. Calculated remodeling and growth indexes showed that restructuring of the vessel wall was not limited to an increase in lumen diameter; there was also an increase in the volume of vascular muscle. These changes were termed hypertrophic outward remodeling (86). Whether the trophic change was due to hypertrophy or hyperplasia of vascular muscle, or both, was not established. These changes in large artery structure were attenuated by neonatal sympathectomy of rats along with the marked attenuation of hypertension.

In contrast to the case of large arteries, there was a clear dissociation between blood pressure and structure of small arteries in Ang II-treated sympathectomized and sham-sympathectomized rats. Ang II treatment resulted in an increase in WLR of small resistance arteries. The extent of structural changes was, however, the same in sympathectomized and sham-sympathectomized Ang II-treated rats despite there being a large difference between their blood pressures. The extent of structural changes in sympathectomized Ang II-treated rats was not related to the blood pressure load. If we were to plot the the blood pressure loads of rats (Fig. 9) against the WLR of their small arteries, a

clear dissociation of the two parameters would be apparent. These findings suggest that Ang II had a direct effect on small artery structure.

The different reponses of small and large mesenteric arteries in Ang II-treated sympathectomized rats may be explained on the basis of the relative distributions of sympathetic innervation and Ang II receptors. This suggestion is based on hemodynamic measurements (88). Indwelling catheters have been placed into large and small mesenteric arteries of rats so that segmental resistances could be measured directly. Injection of norepinephrine into awake rats resulted in uniform constriction of all segments of the mesenteric arterial tree, but the vasoconstrictor action of Ang II was limited to small arteries. These findings suggest that density or sensitivity, or both, of Ang II receptors is greater in small than it is in large arteries, and, therefore, structural changes in small arteries may be the result of a direct trophic action of Ang II. For the development of structural changes in large arteries, on the other hand, the contribution of Ang II-induced hypertension and sympathetic activity may also be required. That Ang II can potentiate sympathetic vasoconstrictor tone by altering the uptake and release of norepinephrine at nerve terminals has been known for some time (36). The present findings suggest that there could be also an interaction between the trophic effects of the two systems.

In regard to large arteries, our findings differ somewhat from the findings of Griffin et al. (33) but are in agreement with those of Levy et al (89), who found outward hypertrophic remodeling of the carotid artery, along with an increase in the number of nuclei, in Ang II-treated rats. Griffin et al. (33) investigated large mesenteric artery (>250 μm outer diameter) structure in Ang II-treated rats using myographic techniques. They found increases in wall thickness and medial wall area of large arteries like we did but no change in lumen diameter.

Consequently, the calculated WLR of arteries was greater. However, Griffin et al. (33) performed their morphometric measurements while the arteries were incubated in a physiologic salt solution and, therefore under active tension. We, in contrast, fixed the arteries at maximal vasodilatation. Another important difference between our results and theirs is the effect of antihypertensive treatment on the development of structural changes in large arteries. Hydralazine treatment in the study of Griffin et al. (33) prevented the development of Ang II-induced hypertension but not the hypertrophic remodeling of arteries, suggesting that the trophic changes were the result of a direct action of Ang II on the vessel wall. In our study, neonatal sympathectomy attenuated both the hypertension and the hypertrophy of large arteries in Ang II-treated rats. Thus, prevention of hypertension is not enough for the attenuation of Ang II-induced hypertrophy of large arteries; abolition of sympathetic activity is also required.

Sympathectomy attenuated the development of structural changes in large arteries but had no effect on the development of structural changes in small arteries of Ang II-treated rats. The trophic effect of Ang II on large arteries appears to be mediated in part through interaction with sympathetic nerves. Structural changes in small arteries, on the other hand, appear to be due to a direct trophic effect of Ang II.

6.4. *Structural vascular changes in hypertension – role of Ang II, dietary sodium supplementation, blood pressure and time*

The findings of this study provide evidence of Ang II-induced hypertension and of the concomitant development of structural vascular changes. A lesser dose of Ang II may lead to the same increase in WLR of resistance arteries as a higher dose as long as the period of treatment

is increased. Subtreshold or near-treshold stimuli, such as 2% NaCl diet and 50 $ng \cdot kg^{-1} \cdot min^{-1}$ Ang II sc, applied simultaneously lead to hypertension and the development of structural vascular changes, demonstrating true synergism. When the WLR of resistance arteries and the BP load over time of several groups of Ang II-treated rats were plotted together on the same graph, a clear dissociation between structural vascular changes and BP was demonstrated, especially in the earliest stages of hypertension.

In the prevoius studies we found that the earliest changes in small and large mesenteric artery structure of rats treated with various doses of Ang II were detectable in the smallest arteries (50 to 100 μm OD), some of which may be best characterized as arterioles. Neonatal sympathectomy had a different effect on the development of structural changes in small and large arteries of rats treated with 200 $ng \cdot kg^{-1} \cdot min^{-1}$ Ang II sc for 4 weeks; the increase in WLR of large arteries was markedly attenuated, along with the attenuation of hypertension, but that of small arteries unaffected (Chapter 5.3.). These findings suggest that a direct trophic effect of Ang II played an important role in the development of structural changes in small arteries, whereas in large arteries structural changes resulted mainly from elevation of BP and sympathetic stimulation.

The findings of the present study provide additional evidence that the earliest structural changes occur in the smallest resistance arteries. An increase in WLR of small but not of intermediate-size arteries was found in rats treated with 50 $ng \cdot kg^{-1} \cdot min^{-1}$ Ang II for 12 weeks and in rats treated with 100 $ng \cdot kg^{-1} \cdot min^{-1}$ Ang II for 4 weeks. The differential segmental arterial effects of Ang II exerts a direct trophic effect on small resistance arteries.

Interestingly, in the present study, there was a trend for increased WLR of small resistance arteries in salt-fed rats. This was achieved with moderate salt supplementation that had no effect on the BP of rats. In past studies, a large amount of salt was fed to rats for 5 to 8 months to produce hypertension and, in some rats, necrotizing arteritis (61). Because of the large amount of obligatory salt intake to which these rats were exposed, the relevance of these experiments to human hypertension is difficult to assess. Another interesting aspect of salt supplementation in our study is that the rats were evenly divided between responders (that is, those that displayed structural vascular changes) and nonresponders. These findings suggest a new definition of salt sensitivity and resistance; rats that respond with an increase in WLR of small resistance arteries to salt supplementation may be termed salt-sensitive and those that do not, salt-resistant. The direct trophic effect of Ang II and, in some rats, of dietary salt supplementation may form the basis of synergism that we found between these two stimuli in producing hypertension and structural vascular changes (details in Chapter 6.1.).

Besides dose dependence, there was also time dependence of the development of structural vascular changes in rats. One half of an effective dose of Ang II produced the same increase in WLR of resistance arteries as the full dose when the time of administration was increased three-fold. Whether this time dependence is due to the cumulative effect of increases in BP or to a cumulative trophic action of Ang II cannot be determined from our data. The concept that a small, initially subpressor stimulus applied for a long period of time may lead to structural vascular changes and hypertension was first promulgated by Lever (32). The present study provides additional data in support of this hypothesis. Time is certainly a factor in the development of human hypertension. Chronic hypertension is frequently ushered in by years of

borderline hypertension. Essential hypertension typically becomes established in the third and fourth decade of life. During the developmental stage of hypertension, the pressor stimulus may be so small as to be virtually undetectable in comparison with that in normotensive group of subjects.

The nature and development of structural vascular changes have been the focus of numerous investigations into the pathogenesis of hypertension because without these changes chronic hypertension does not occur (13, 27, 28, 29, 30). The importance of the present study lies in the analysis of the relationship that exists between BP and structural vascular changes in this model of hypertension. When BP load over time and WLR of small resistance arteries of the various treated groups of rats were plotted on the same graph, a clear dissociation between the two parameters was observed in the earliest stages of the hypertensive process. The dissociation between BP load and vascular structure in the earliest stages of hypertension suggest that a direct trophic effect of Ang II, in addition to its pressor one, is contributing to the development of structural vascular changes.

In summary, the development of structural vascular changes in Ang II-treated rats is dose- and time-dependent. The earliest changes are detected in small resistance arteries and in arterioles. The dissociation between BP load and WLR of mesenteric resistance arteries in the earliest stages of hypertension suggest that direct trophic stimulation of vascular wall by Ang II contributes to the development of structural vascular changes. Dietary sodium supplementation potentiates the trophic vascular and pressor effect of Ang II.

6.5. *Hypertension and progression of the kidney disease in patients with IgA nephropathy*

Hypertension in IgA nephropathy is a typical example for secondary human hypertension. Hypertension is a bad prognostic factor in IgA nephropathy (90, 91). Ang II seems to have an important role in the development of hypertension and in the progression of the kidney disease in IgA nephropathy (42-46).

As casually measured high blood pressure seems to accelerate the decline of renal function in IgA nephropathy (34, 36-40), it is of interest to study the 24-hour blood pressure of normotensive and hypertensive IgA nephropathy patients and to analyse the different parameters of ambulatory blood pressure measurements. With the increasing availability of ABPM it became possible not only to measure the blood pressure during 24 hours, but also to identify between „dippers" and „non-dippers", „white-coat hypertensives" and „real" hypertensives and to control better the treatment of high blood pressure during day- and night-time (94-98). In early IgA nephropathy ambulatory blood pressure is already slightly elevated and diastolic left ventricular malfunction can be detected (90).

According to our results, most of normotensive IgA nephropathy patients have normal diurnal blood pressure variation. As in most types of secondary hypertension (91), in hypertensive IgA nephropathy patients the diurnal blood pressure rhythm has disappeared. Eighty-two per cent of the normotensive IgA nephropathy patients were classified as „dippers", which is similar to the percentage of "dippers" in the normotensive population with no kidney disease. Ninety-three per cent of hypertensive IgA nephropathy patients were classified as „non-dippers", with no decrease or, even, some increase in nocturnal blood pressure.

„White-coat hypertension" has been described in mild forms of essential hypertension (48, 99). We observed the same phenomenon in 18% of our normotensive IgA nephropathy patients, which is very similar to the prevalence of „white-coat hypertension" in patients with mild essential hypertension. Furthermore, we observed the „white-coat phenomenon" in 20% of our treated hypertensive IgA nephropathy patients, too. If we don't count on this phenomenon, we may treat normotensive patients with antihypertensive drugs with no purpose or we may overtreat hypertensive patients. Such treatment is not innocuous, it may cause hypotension and hypoperfusion of the glomerulonephritic kidneys, which may accelerate the decline of renal function. Antihypertensive drugs are expensive and labeling patients as hypertensive might be a psychic burden to them.

Treated hypertensive and normotensive IgA nephropathy patients had similar day-time blood pressure values, but the nigh-time blood pressure was decreased in normotensives and unchanged in hypertensives. It means that hypertensives had higher than normal night-time blood pressure and mean 24-hour blood pressure. This may explain the faster deterioration of renal function in these treated hypertensive patients.

The progression of the kidney disease seemed to be faster in „non-dipper" than in „dipper" normotensive IgA nephropathy patients, too. This underlines the importance of the night-time blood pressure in the development of target organ damage. The higher blood pressure load caused by higher night-time blood pressure (100) seems to be important in the decline of renal function in both hypertensive and normotensive IgA nephropathy patients.

The progression of the kidney disease was faster in „white-coat hypertensive" patients than in „real" normotensive patients, too. It has

been shown, that office hypertension is not an innocent blood pressure variant in essential hypertension (101). This seems to be the case in IgA nephropathy, too. The decline of renal function in these patients seems to be faster, and most of these patients may develop hypertension later on. According to our results, the lack of the blood pressure „dip" at night seems to accelerate the decrease in renal function in IgA nephropathy patients. Early antihypertensive therapy providing adequate 24 hour blood pressure control - eventually even mimicking the normal nocturnal decrease of blood pressure - may slow the development of end-stage renal failure in these patients. ACEI and CCB treatment seems to be not effective to resettle the diurnal blood pressure variation. The percentage of „white-coat hypertensives" among normotensive IgA nephropathy patients and mild hypertensives with normal or nearly normal renal function is similar and it may accelerate the progression of the kidney disease.

It was more than 100 years ago when the pressor properties of renal cortical extracts were dicovered and the era of hypertension research began. Although more than 100 years of intense research have produced a large body of new information, hypertension still continues to be a major problem of medicine. In the second half of the twentieth century the control of hypertension in North America, Western Europe, Japan and Australia has improved considerably (3). At the same time, progressive decrease in cardiovascular mortality was seen in these regions (102). On the other hand, a „second wave" epidemic of cardiovascular disease is now flowing through the former socialist republics and the developing countries (103). Elevated blood pressure has a central role in the pathogenesis of these diseases (104). According to the above, hypertension is still one of the biggest challanges facing researchers, medical practitioners and even public health authorities.

Understanding the mechanisms leading to the developmet of hypertension in animal models and analysing the onset of hypertension with its consequences in humans may help us in the future to better handle this disease affecting millions of people worldwide.

7. NEW RESULTS

1. *The effect of dietary sodium supplementation on Ang II-induced hypertension in rats*

Short term, high-sodium diet increases vasoconstrictor responses to Ang II and periarterial nerve stimulation, and in combination with Ang II treatment further potentiates vasoconstrictor responses to Ang II (synergism).

Long term, Ang II in subpressor doses increases WLR of small resistance arteries significantly without a rise in the blood pressure. Ang II and high-sodium diet raises SBP significantly and increases small artery WLR further.

The interaction between Ang II and high-sodium diet appears to be specific to Ang II and is occuring on the vascular level.

2. *Effect of neonatal sympathectomy on development of Ang II-induced hypertension in rats*

Neonatal sympathectomy prevents autopotentiation of vasoconstrictor responses in the early stages of Ang II administration and the generalized increase in mesenteric vasoactivity during chronic Ang II administration.

As a functional consequence of the above, sympathectomy prevents the development of chronic hypertension in Ang II-treated rats.

Sympathectomy does not prevent the structural vascular changes of small arteries induced by chronic Ang II administration, supporting a direct trophic vascular effect of the agonist.

3. *Effect of neonatal sympathectomy on the development of structural vascular changes in Ang II-treated rats*

Sympathectomy alone increases the lumen and reduces the WLR of first- and second-order branches of the superior mesenteric artery in rat (hypotrophic outward remodeling).

Ang II treatment increases the dimensions, wall thickness, and wall area of first- and second-order arteries (hypertrophic outward remodeling) and the WLR of small resistance arteries in sham-sympathectomized rats.

Neonatal sympathectomy attenuates the development of structural changes in large arteries but has no effect on the development of structural changes in small arteries of Ang II-treated rats. Hypertension and sympathetic innervation appear to be contributing to the development of structural changes in large arteries of Ang II-treated rats. Structural changes in the small arteries appear to be due to a direct trophic effect of Ang II.

4. *Structural vascular changes in hypertension – role of Ang II, dietary sodium supplementation, blood pressure and time*

The development of structural vascular changes in Ang II-treated rats is dose- and time-dependent.

The earliest changes are detected in small resistance arteries and in arterioles.

Ang II treatment causes hypertrophy of the arterial smooth muscle cells.

There is a dissociation between BP load and WLR of mesenteric resistance arteries in the earliest stages of hypertension suggesting that direct trophic stimulation of vascular wall by Ang II contributes to the development of structural vascular changes.

Dietary sodium supplementation potentiates the trophic vascular and pressor effect of Ang II.

5. *Hypertension and progression of the kidney disease in patients with IgA nephropathy*

In normotensive IgA nephropathy patients the diurnal BP variation is preserved. Most of the hypertensive IgA nephropathy patients have no diurnal blood pressure variation.

The lack of the normal diurnal blood pressure variation seems to accelerate the progression of IgA nephropathy.

„White-coat hypertension" occurs in IgA nephropathy patients in similar prevalence as in mild essential hypertensives.

„White-coat hypertension" seems to accelerate the progression of the kidney disease.

ACEI treatment in monotherapy or in combination with CCBs is able to normalise the day-time blood pressure, but not the night-time blood pressure of hypertensive IgA nephropathy patients. ACEI and CCB treatment is not able to resettle the normal diurnal blood pressure variation in IgA nephropathy patients.

8. LIST OF PUBLICATIONS

8.1. *Publications*

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Synergistic vascular effects of dietary sodium supplementation and angiotensin II administration
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8.3. *Abstracts*

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