Acta path. microbiol. scand. Sect. A, *83:* 661–668, 1975

THE EFFECT OF ANGIOTENSIN INFUSION, SODIUM LOADING AND SODIUM RESTRICTION ON THE RENAL AND CARDIAC ADRENERGIC NERVES

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Ljungqvist, A. The effect of angiotensin infusion, sodium loading and sodium restriction on the renal and cardiac adrenergic nerves. Acta path. microbiol. scand. Sect. A, *83:* 661–668, 1975.

The renal and cardiac adrenergic nerve patterns in rats infused with large and small amounts of angiotensin and in rats given NaCl plus DOCA, NaCl alone, and salt-free diets were examined by the histochemical fluorescence method. Infusion of small amounts of angiotensin led to a persistent blood pressure elevation whereas infusion of large amounts of angiotensin resulted in a transient rise in blood pressure, probably due to the development of tachyphylaxis. Nerve patterns were found to be normal in angiotensin-infused rats and in rats given NaCl. In rats given NaCl plus DOCA and in rats subjected to salt restriction, a partial or complete disappearance of the transmitter of the adrenergic nerve terminals were recorded. The findings suggest that angiotensin in itself is incapable of inducing visible alterations in the transmitter content of the terminals. The findings agree with the view that angiotensin potentiates a norepinephrine depletion of the terminals during sympathetic activity, since it can be assumed that increased plasma angiotensin levels as well as various degrees of increased sympathetic tonus were present in the rats subjected to salt restriction. The similar effect on the nerve terminals produced by the combined NaCl and DOCA administration is consistent with earlier reports of an increased turnover of norepinephrine in animals thus treated.

Key words: Angiotensin infusion; sodium; renal nerves; cardiac adrenergic nerves.

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Received 6.vi.75 Accepted 6.vi.75

It is known that an increased release of renin from the kidney can be induced by stimulation of the renal sympathetic nervous system (15, 2). This may be the result of a direct adrenergic stimulation of the renin-producing juxtaglomerular cells since these are surrounded by adrenergic nerve terminals (19).

In recent studies of renal hypertensive rats, a partial or complete disappearance of the transmitter content from the sympathetic nerve terminals was observed in the clipped kidney during the early phase of hypertension (13, 12). Since the plasma angiotensin level is known to be elevated during the early phase of renovascular hypertension and since angiotensin is known to potentiate the release of noradrenalin from the terminals of stimulated sympathetic nerves (10, 15), it was

suggested that the observed alterations were the result of an angiotensin effect on a hyperactive intrarenal sympathetic nervous system.

If this explanation holds true, a primary activation of the sympathetic innervation of the juxtaglomerular cells would be involved in the development of renovascular hypertension. A possible, alternative explanation would be that the renin-angiotensin-aldosterone system was activated by extranervous mechanisms and that the consequent alterations in the intrarenal sodium/water balance exerted a direct effect on the nerve terminals.

The present investigation was undertaken with a view to assessing the influence of angiotensin and variations in sodium load on the juxtaglomerular adrenergic nerve terminals.

MATERIAL AND METHODS

Angiotensin Infusion

Twenty-four female Sprague-Dawley rats weighing between 180 and 200 g were used for this part of the study. The rats were anaesthetized by intraperitoneal administration of Nembutal. The right carotid artery was exposed and cannulated for continuous recording of the arterial blood pressure. The left carotid artery was exposed and cannulated for intra-arterial infusion of angiotensin (Hypertensin, Ciba). Since highly different amounts of angiotensin were to be injected into the various rats at a constant rate of 0.15 ml of the solution per minute (see below), samples of angiotensin solutions of different concentrations were prepared. The rats were divided into three groups as follows:

Group I: Seven rats into which between 50 and 75 μg of angiotensin was infused over 5–30 minutes.

Group II: Nine rats into which between 0.75 and 1.5 μg of angiotensin was infused over 10–15 minutes.

Group III: Eight control rats which were given an intra-arterial infusion of 0.15 ml/min of isotonic saline over a period of 10–15 minutes.

The kidneys of each rat were removed while the animal was still under infusion. With a view to comparison, the hearts were also removed and the organs processed as described below for examination of their adrenergic innervation patterns.

Salt Loading and Salt Restriction

Sixty female Sprague-Dawley rats were used in these experiments. At the beginning of the experiments, the weight of the rats ranged from 180 to 200 g. Blood pressure measurements were performed by the tail plethysmographic method. Before the experiments started, all rats had a blood pressure well below 140 mm Mg and were therefore regarded as normotensive. The rats were divided into the following groups:

Group IV: Twenty rats in which DOCA-pellets were implanted intramuscularly. The rats were given a standard laboratory diet containing 0.4 per cent NaCl and a 2 per cent aqueous solution of NaCl *ad libitum* as drinking fluid.

Group V: Ten rats which were kept on the same food and drinking fluid as the rats of group IV, but in which no DOCA-implantations were made.

Group VI: Twenty rats which were given the above laboratory diet from which the NaCl was withdrawn. Distilled water was given as drinking fluid.

Group VII: Ten control rats which were given the standard laboratory food and tap water.

Preliminary studies had suggested a certain mortality in rats given DOCA + salt and in rats subjected to salt restriction. Therefore, these two groups of rats (groups IV and VI) were doubled when compared with the other groups (groups V and VII). In group IV, however, only two rats died during the experiments and only one in group VI.

The rats in groups IV–VII were followed by daily weight and blood pressure determinations for one week. Hypertension was considered to have developed when the blood pressure steadily exceeded 140 mm Hg, provided that this included a rise by 15 per cent, at a minimum of the initial level. On the 8th day, the rats were anaesthetized by ether and the kidneys and hearts were removed for examination. The rats were then killed by an overdosage of ether.

All kidneys and hearts were examined with a view to their adrenergic innervation patterns, using the histochemical fluorescence method for demonstration of biogenic mono-amines (for references see 4, 9). For this purpose, pieces of tissue from each organ were rapidly frozen in liquid propane cooled by liquid nitrogen. This was followed by freeze-drying of the specimens at −30° C upon which they were exposed to formaldehyde-gas for 1 hour at +80° C (water content of formaldehyde-powder 0.6 per cent). Some pieces from each organ were processed directly as described, whereas other pieces were first incubated in α-methyl-norepinephrine of various concentrations (5×10^{-4} M and 5×10^{-5} M), as previously described (16).

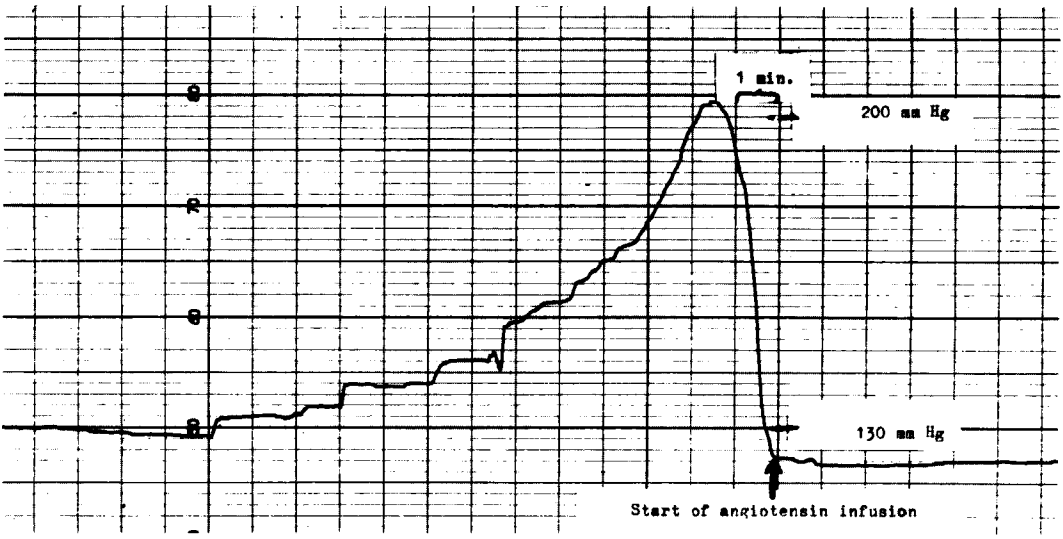

Fig. 1. Recording of the blood pressure response to infusion of high doses of angiotensin into the rat (3.5 μg/min during 18 minutes). There is an early rise in blood pressure, followed by a slow return to normal levels. C.f. Fig. 2.

Subsequently, all specimens were vacuum embedded in paraffin and 5–6 μ thick sections were cut and mounted in nonfluorescent medium (Entellan, Merck) to which xylol was added. The sections were examined in the fluorescence microscope. They were labelled according to a codified system and were examined without knowledge of grouping and clinical picture.

RESULTS

Angiotensin Infusion

As regards the control rats (group III), no effects on the blood pressure were recorded. In the rats infused with a highly concentrated angiotensin solution (group I) there was a rapid rise in blood pressure which, however, gradually returned towards normal levels (Fig. 1). In the rats infused with an angiotensin solution of low concentration (group II) there was a rapid rise in blood pressure which remained elevated until the animals were killed (Fig. 2).

Examinations of the hearts and kidneys in the fluorescence microscope revealed no differences between the various groups of rats. In all kidneys, networks of yellowish-green fluorescent varicose fibres of the appearance typical of adrenergic nerve terminals were seen to accompany the arterial arborization up to the postglomerular capillaries (Fig. 3 B). No fibres were encountered in the glomeruli or along the veins. This picture is identical with the normal intrarenal adrenergic innervation pattern described previously (14, 19).

In the hearts, an entirely normal innervation pattern as that described by *Winckler* (18) was also observed. Thus, fluorescent varicose fibres encirceled the intramyocardial arteries and arterioles and were encountered in large numbers in the interstitial tissue between the muscle fibres (Fig. 4 B).

Any differences in nerve patterns in sections from pieces processed directly and from pieces incubated in α-methyl-norepinephrine before processing were not observed in any of the animals.

Salt Loading and Salt Restriction

In the rats subjected to salt restriction (group VI) and in the control rats (group VII) the same increase in weight was recorded during the experimental week (Table 1) and the blood pressures were normal. In the salt-loaded animals, whether DOCA was given (group IV) or it was not given (group V), a significant decrease in body weight was recorded (Table 1). Blood pressure elevation

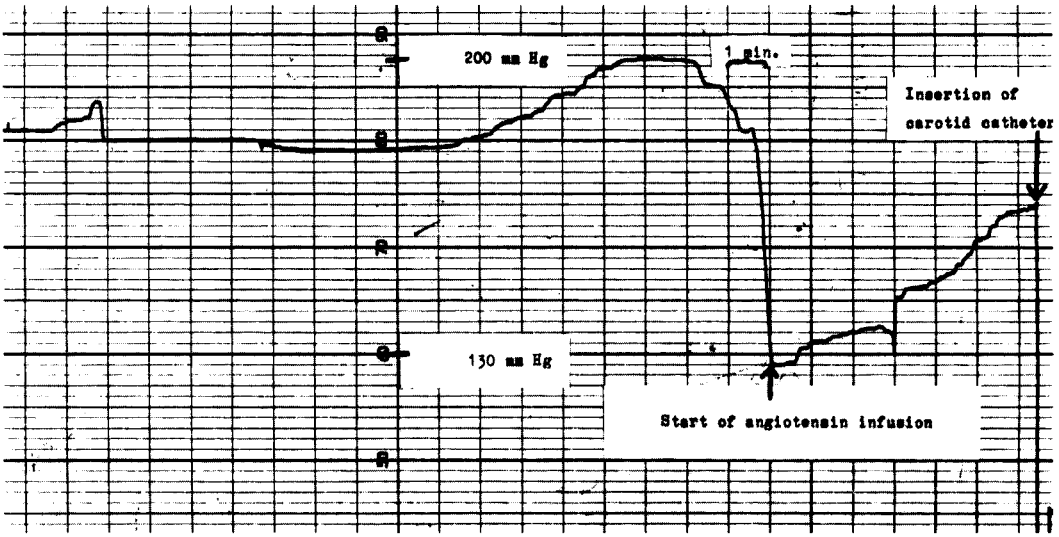

Fig. 2. Recording of the blood pressure response to infusion of small doses of angiotensin (0.075 μg/min during 18 minutes). Insertion of the carotid artery catheter usually caused a rise in blood pressure and the infusion of angiotensin did not start until the blood pressure had normalized. The infusion resulted in an early rise in blood pressure which persisted at this higher level until the organs were removed. C.f. Fig. 1.

TABLE 1. *Body Weights and Blood Pressures at Start and End (1 Week Later) of the Experiments*

Group	No. of rats	Body weight		Blood pressure	
		Start	End	Start	End
IV	18	190 ± 5	155 ± 25	90 ± 5	175 ± 20
V	10	195 ± 5	125 ± 5	90 ± 5	80 ± 5
VI	19	200 ± 5	205 ± 10	90 ± 10	110 ± 15
VII	10	190 ± 5	200 ± 5	90 ± 10	100 ± 15

Rats in groups IV and V were salt loaded, those in group IV being supplemented with DOCA. Rats in group VI were given a salt-free diet and distilled water and those in group VII were controls which had standard laboratory diets and tap water. The figures are mean values ± SD.

TABLE 2. *Fluorescence Reaction Indicative of the Presence of Adrenergic Transmitter Substance in the Nerve Terminals in the Heart and Kidneys of Rats Given NaCl and DOCA (Group IV), NaCl Alone (Group V), Salt-Free Diets (Group VI) and Normal Standard Diets (Group VII)*

Group	No. of rats	Heart			Kidney		
		normal	reduced	absent	normal	reduced	absent
IV	18	0	3	15	0	10	8
V	10	10	0	0	8	2	0
VI	19	0	7	12	7	5	7
VII	10	10	0	0	10	0	0

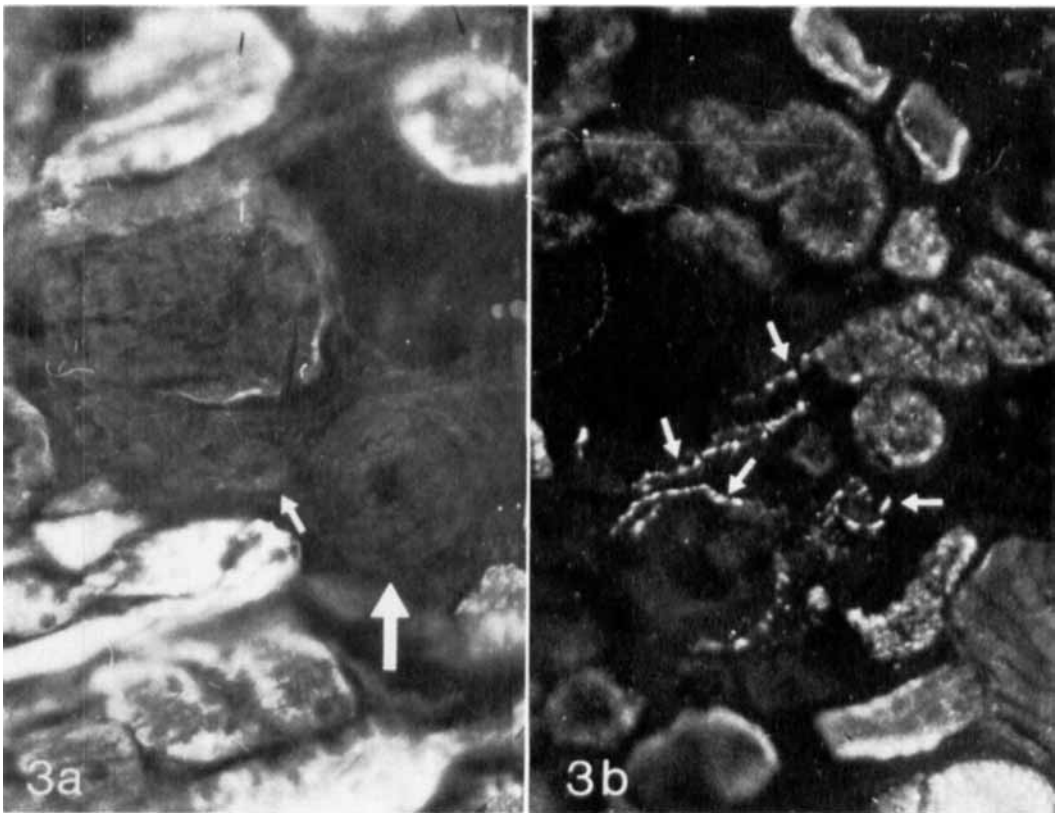

Fig. 3 A. Fluorescence microphotograph of the kidney of a rat that had been subjected to combined salt-DOCA treatment. An intrarenal artery (large arrow) and arteriole (small arrow) is seen. The latter leads to a glomerular tuft. Autofluorescence of tubules; no fluorescent varicose fibres are seen. × 350.

Fig. 3 B. Fluorescence microphotograph of a section from the same kidney as that depicted in Fig. 3 A after incubation with α-methyl-norepinephrine. A normal nerve pattern with fluorescent varicose fibres around arteries and arterioles is seen (arrows). Autofluorescence of tubules. Dark area in centre, left corresponds to a glomerulus. × 350.

occurred only in the rats given both salt and DOCA (group IV), but in these it was regular and often considerable (Table 1). This blood pressure elevation usually became evident as early as on the second day of the treatment, upon which it gradually increased.

The findings obtained by examination of the adrenergic innervation patterns of the hearts and kidneys in the various groups of rats are listed in Table 2. It can be seen that the control rats (group VII) displayed the above described normal patterns both in hearts and kidneys. In the experimental groups, no significant alterations were observed in the salt-loaded rats not given DOCA (group V); only in two of these rats did the non-incubated kidney specimens show a certain reduction in the density of the fibre networks along the intrarenal arterioles and this was asociated with a comparatively weak fluorescence of the fibres along the finer intrarenal arterial branches. These nerve alterations are referred to as a "reduced" pattern. In the incubated specimens from these two kidneys, the nerve patterns were entirely normal.

Significant alterations of the intramyocardial and intrarenal adrenergic nerve patterns were observed in the non-incubated specimens from the salt-loaded rats given

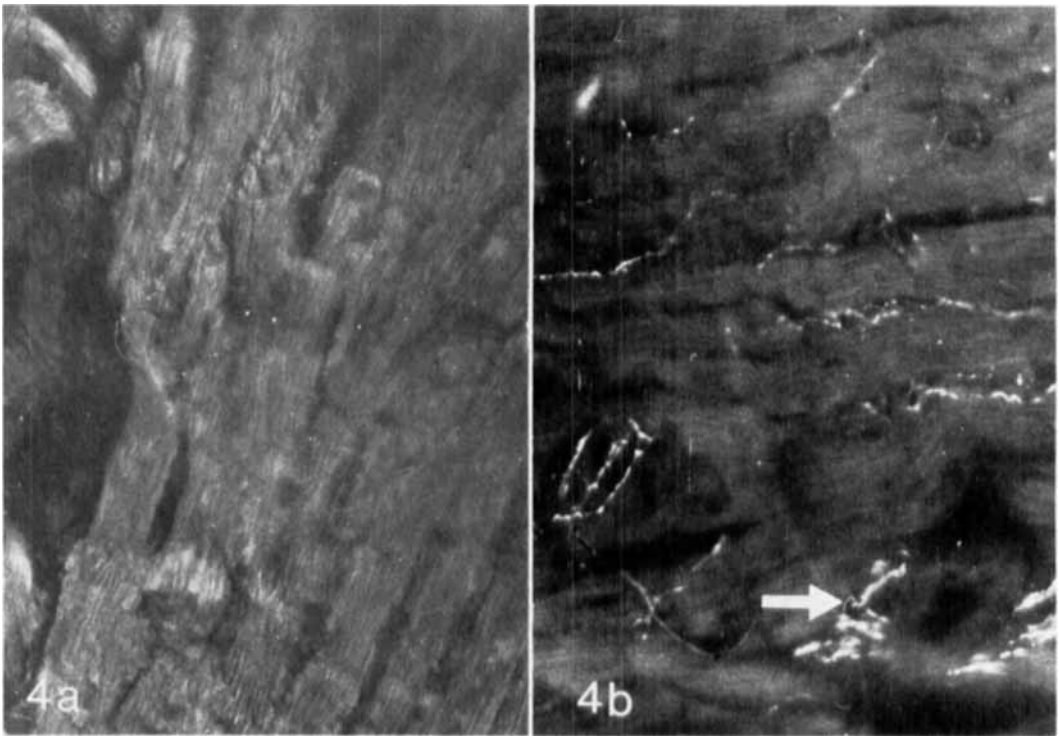

Fig. 4 A. Fluorescence microphotograph of the myocardium (left ventricle) of a rat that had been subjected to combined salt-DOCA treatment. The dark area to the left corresponds to an artery branch. No fluorescent varicose fibres are seen. × 350.

Fig. 4 B. Fluorescence microphotograph of section from the same heart as in Fig. 4 A after incubation with α-methyl-norepinephrine. A normal nerve pattern with fluorescent varicose fibres around an artery (arrow) and between the muscle bundles is seen. × 350.

DOCA (group IV) and from the rats subjected to salt restriction (group VI). As regards the former rats, all hearts but one were completely devoid of fluorescent varicose fibres (Fig. 3A) and in eight of these animals such fibres were also absent in the kidneys (Fig. 4A); in 10 rats, the intrarenal nerve pattern was reduced. In the rats subjected to salt restriction, the nerve pattern was destroyed in twelve hearts and reduced in the remaining seven. As regards the kidney, the corresponding figures were seven and five, leaving seven kidneys with a normal nerve pattern.

As regards the incubated material, all sections of heart and kidney from rats in groups IV and VI showed normal nerve patterns (Figs. 3 B and 4 B).

DISCUSSION

The present investigation was initiated by the recent finding that, in rats made hypertensive by the production of unilateral renal artery stenosis, the early phase of the blood pressure elevation was associated with a diappearance of the adrenergic transmitter from the nerve terminals at the juxtaglomerular level in the stenosis kidney (13, 12). Since this alteration coincided with the period during which the plasma angiotensin level is known to be increasing and angiotensin is known to potentiate the release of noradrenalin from the terminals of stimulated sympathetic nerves (10, 15), it was pointed out that the nerve alterations might reflect a sympathetic overactivity at the juxtaglomerular level which

would thus precede the development of hypertension and therefore possibly be of pathogenetic significance. An alternative theory would be that the renin-angiotensin-aldosterone system might be activated by extranervous mechanisms and that the consequent alterations in the intrarenal sodium/water balance would exert a direct effect on the adrenergic nerve terminals.

It was found in the present investigation that angiotensin alone is not capable of inducing visible alterations in the transmitter content of the nerve terminals, whether given in physiological pressor doses or in non-physiological, large amounts. The failure of angiotensin to induce a sustained blood pressure elevation if infused in large amounts has been demonstrated by others (1) and has been ascribed to the development of tachyphylaxis (3).

Alterations in the nerve patterns were observed, however, if salt loads of the animals were varied. These alterations were not restricted to the juxtaglomerular areas in the kidney but involved the entire organ, as well as the heart, suggesting that they were generalized.

It has previously been shown that NaCl + DOCA treatment of rats will lead to a decreased norepinephrine storage by the sympathetic nerves of various organs and that this occurs early during the treatment while the animals are still in the prehypertensive phase (5, 6, 11). Later observations suggest that the sodium ion is the regulating factor and that the decreased norepinephrine storage is due to an increased turnover of the substance, indicating an increased release of norepinephrine from the nerves (7, 8). These observations are in good agreement with the present findings of a partial or complete disappearance of fluorescent adrenergic nerve terminals in the heart and kidneys of rats given NaCl and DOCA.

de Champlin et al. (7) also found that administration of NaCl (or DOCA) alone was associated only with a minor decrease in the norepinephrine storage capacity. This again is in agreement with the present findings of normal nerve patterns in the NaCl-treated rats, but throws some doubt on the hypothesis that the sodium ion is of importance as a regulating factor in the peripheral turnover of norepinephrine. Still, it cannot be ruled out that, in the absence of the sodium retaining hormone DOCA, the sodium load was simply too low to produce visible alterations.

In agreement with the hypothesis that sodium is capable of regulating the turnover of norepinephrine of the sympathetic nerves, *de Champlin et al.* (7) recorded an increased norepinephrine storage by the nerves during sodium restriction. This seems to contradict the findings in the present study where partial or complete disappearance of the adrenergic nerve terminals in the heart and kidneys was observed in many animals subjected to NaCl restriction. Animals thus treated, however, are known to have elevated plasma renin and plasma angiotensin levels and angiotensin is known to potentiate the release of norepinephrine from the terminals of stimulated nerves (10, 15). Any tendency of the sodium deficiency to induce an increased storage of norepinephrine by the nerve terminals is therefore counteracted by the elevated plasma angiotensin, provided that the sympathetic nerves are stimulated. Thus, whether one or the other factor will predominate in an animal depends probably upon the state of activity of the sympathetic nervous system in the animal concerned and, in this respect, individual variations are to be expected in laboratory animals subjected to experimental procedures. This would explain the variability in the appearance of the nerve patterns in the present animals subjected to NaCl restriction.

This study was supported by the *Swedish Medical Research Council* (Project No. B75-12X-716).

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