3. B. A. Vitsyn and E. M. Blagitkov, Artificial and Spontaneous External Intestinal Fistulas [in Russian], Novosibirsk (1983), pp. 29-113.
4. E. I. Gal'perin, M. I. Semendyaeva, and E. A. Neklyudova, Hepatic Failure [in Russian], Moscow (1978), pp. 16-29.
5. Yu. Ya. Gritsman and A. I. Borisov, Postoperative Intestinal Fistulas [in Russian], Moscow (1972), pp. 15-21.
6. D. P. Chukhrienko and I. S. Belyi, External Intestinal Fistulas [in Russian], Kiev (1975), pp. 29-65.
7. R. D. Chkheidze, Éksp. Khir., No. 4, 42 (1975).
8. D. B. Allardyce, Am. J. Surg., 145, 593 (1983).
9. G. Dietz, M. Wicklamayer, and H. Mehnert, Parenteral Nutrition, New York (1976), pp. 17-30.
10. R. C. Halversen, H. H. Hogle, and R. C. Richards, Am. J. Surg., 118, 968 (1969).

MORPHOLOGY OF THE ADRENERGIC AND CHOLINERGIC INNERVATION OF THE KIDNEY IN RATS WITH SPONTANEOUS AND GOLDBLATT HYPERTENSION

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A special pattern of functioning, or "resetting" of the kidney is nowadays regarded as one of the most important steps in the pathogenesis of arterial hypertension [1, 2, 7]. Although the importance and pathogenetic role of "resetting" as a whole are not disputed, the intrarenal and extrarenal mechanisms of its realization have not been adequately studied. For instance, very little is known about the role of the intramural nerves in the development of "resetting" or in the pathogenesis of hypertension in general, even though their regulatory effect on various renal structures has been proved. In some investigations the development of spontaneous hypertension in rats was delayed after division of the renal nerves [4, 11, 12, 17], but neurophysiological investigations disagree in their conclusions regarding the importance of the renal nerves in the formation and prolongation of this hypertension. For instance, it was shown by recording activity of efferent sympathetic nerves [8, 14] that its level is much higher in spontaneously hypertensive rats (SHR) than in control animals. However, no differences in activity of the sympathetic renal innervation have been found between SHR and the normotensive control [6, 16]. Denervation of the kidneys in ischemic renal (Goldblatt) hypertension was shown that their nerves participate in the development of this form of hypertension also [5], although only the ischemic kidney has been studied by morphological methods, and no publications dealing with the state of the nerves in the contralateral "intact" kidney could be found (except [3]).

In accordance with the facts described above, it was decided to undertake a morphological study of the adrenergic and cholinergic nerves of the kidney at different stages of spontaneous and ischemic renal hypertension in rats.

EXPERIMENTAL METHOD

Experiments were carried out on male SHR rats aged 4, 8, 20, and 27 weeks, weighing on average 49 × 2, 222 × 6, 291 × 15, and 427 × 26 g. Normotensive rats (NTR) of the WKY line (Wistar-Kyoto), of the same sex and age, served as the control. Ischemic renal hypertension (bilateral, according to Goldblatt) was produced in inbred Wistar rats (from the Stolbovaya nursery, Academy of Medical Sciences of the USSR) by constriction of the left renal artery. The nonischemic, "intact," kidney was investigated. In these experiments intact rats of the same line, chosen by sex and age, served as the control. The investigation was conducted 4, 8-9, 20-22, and 27-28 weeks after the beginning of the experiment. The systolic arterial

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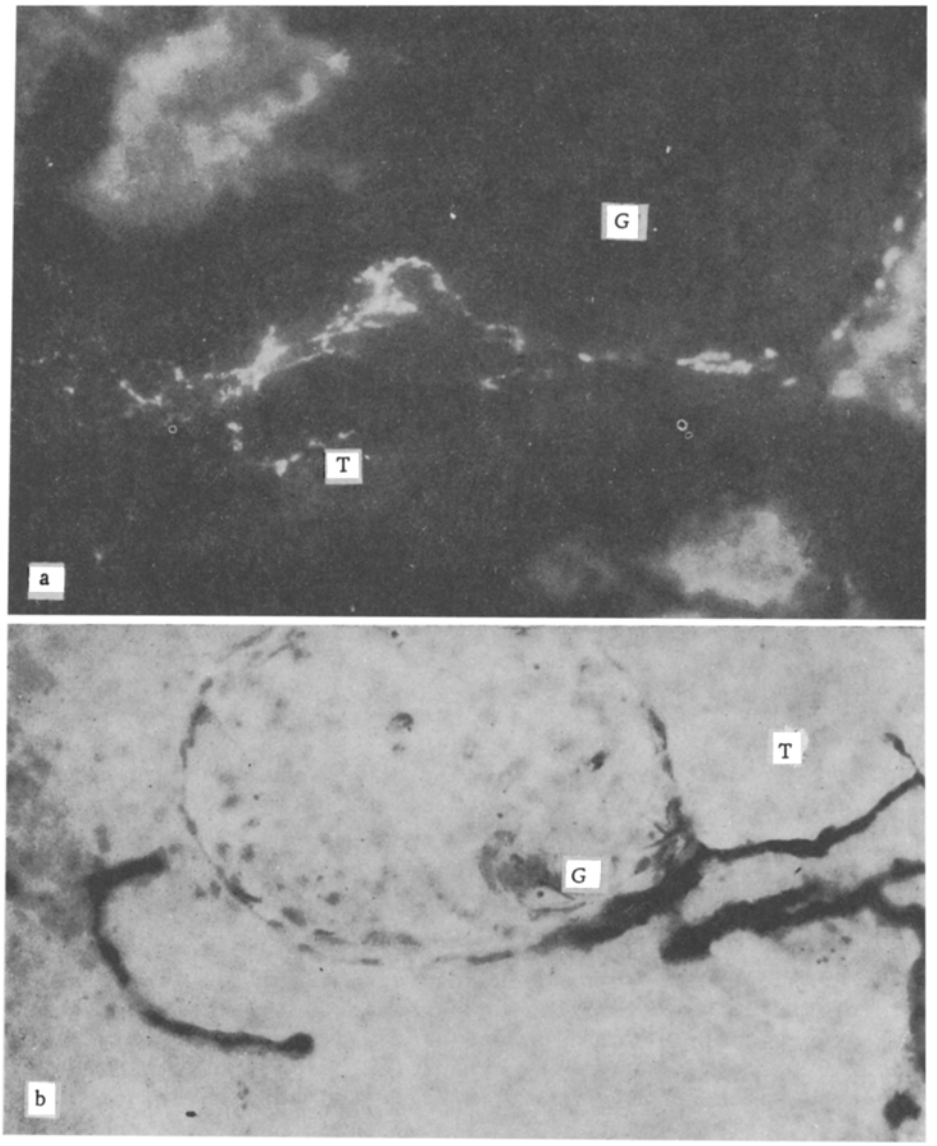

Fig. 1. Innervation of kidney of a spontaneously hypertensive rat: a) adrenergic innervation of vascular pole of glomerulus (G) and tubule (T), method of Torre and Surgeon, 360×; b) cholinergic innervation of vascular pole of glomerulus and tubule, method of Karnovsky and Roots, 400×.

pressure (BP), measured in the caudal artery by a plethysmographic method, without anesthesia, was 160 ± 6 mm Hg for SHR and 112 × 4 mm Hg for NTR. These parameters in Goldblatt hypertension were 156 ± 3 mm Hg in the experiment and 112 ± 2 mm in the control. At each period of investigation, five or six experimental and control animals were killed under superficial ether anesthesia. The adrenergic nerves of the kidney were revealed by a luminescence-microscopic method [15], using glyoxylic acid (Fig. 1a). Cholinergic (cholinesterase-positive) nerves of the kidney were revealed by histochemical detection of cholinesterase by the method in [9] (Fig. 1b). Innervation indices (II) of the vascular pole of the glomeruli with the corresponding juxtaglomerular apparatus (JGA) were determined by calculating the fraction of innervated structures [3]. Because of the lower density of innervation of the tubules, their II were calculated per unit area of median section of the kidney, which was determined planimetrically. When necessary, the innervated structures were identified in serial sections stained with hematoxylin and eosin.

EXPERIMENTAL RESULTS

In 4-week-old SHR the adrenergic II of the vascular pole of the glomeruli and JGA was a little higher than its value in the control (Fig. 2). Later these differences increased, so

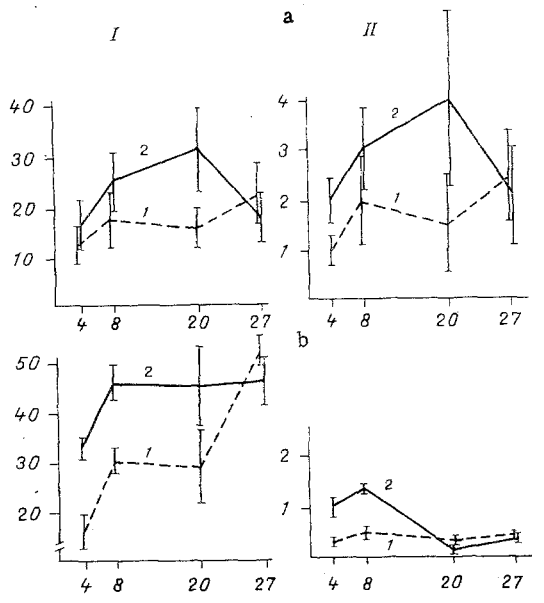

Fig. 2

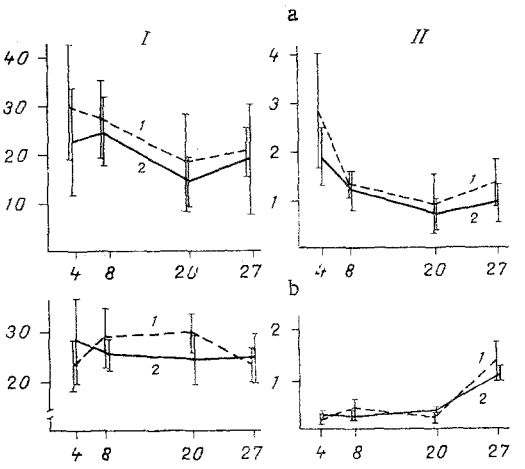

Fig. 3

Fig. 2. Time course of adrenergic (a) and cholinergic (b) II in spontaneous hypertension. Abscissa, age of animals (in weeks); ordinate, II (in %). I) Vascular pole, JGA; II) tubules. Continuous line represents experiment; broken line, control.

Fig. 3. Time course of adrenergic (a) and cholinergic (b) II in ischemic renal hypertension. Abscissa, duration of experiment (in weeks). Remainder of legend as to Fig. 2.

that in 22-week-old SHR its level was about twice as high as in the control. Later still, in the period of formation of nephrosclerosis, the number of glomeruli with an innervated vascular pole and JGA cells decreased, returning to values obtained in SHR aged 4 weeks. The same time course of the adrenergic II was observed in the tubules. The cholinergic II of the vascular pole of the glomeruli and JGA in SHR at first considerably exceeded the value of this parameter in the control, but later the differences diminished on account of the fact that it remained at the same level in the hypertensive rats, but increased in the control, and in rats aged 27-28 weeks, the differences disappeared completely. The cholinergic II of the tubules in young SHR was considerably higher than that in the control, but later the differences diminished.

In ischemic renal hypertension (the "intact" kidney) the adrenergic II of the vascular pole of the glomerulus and JGA, after hypertension lasting 4 weeks, showed a very slight tendency to decrease, but throughout the rest of the experiment it was indistinguishable from the control value (Fig. 3). II of the tubules behaved in the same way. The cholinergic II of the vascular pole of the glomeruli and JGA in the 4th week of the experiment had only a very slight tendency to increase, and later it did not differ from the control. The cholinergic II of the tubules likewise showed no significant differences from the control at all times of development of hypertension.

Thus during the formation and subsequent development of genetically determined spontaneous hypertension, both adrenergic and cholinergic II of the principal structures of the kidney were significantly increased, whereas in ischemic renal hypertension (in the "intact" kidney) these parameters were virtually indistinguishable from normal. The changes found in the number of nerve structures in SHR could hardly be due to a true increase in their number. They were most likely due to a change in the "threshold of detectability," resulting from an increased content of neurotransmitter. The increase in the number of nerves with rich reserves of neurotransmitters in the kidney indicates without doubt that they participate in the development of this pathology.

Those differences which do exist in the time course of the adrenergic and cholinergic innervation in SHR are evidence that the role of each type of innervation of the kidney in its changes differs in different phases of the disease. Meanwhile the time course of II of the

glomeruli and tubules for nerve structures of the same type was virtually the same, evidence of the balanced state of the innervation within the nephron and of the preservation of glomerular-tubular balance throughout the experiment.

Under "resetting" conditions, when the osmoconcentrating function of the kidney is under strain, in order to maintain glomerular-tubular and tubular-tubular balance, strengthening not only of humoral, but also of nervous influences on the glomerular hemodynamics and tubular processes is necessary. The twofold or more increase in II is evidence that the role of the renal nerves in the development of spontaneous hypertension is much more important than was hitherto considered. This is in agreement with conclusions drawn from investigations into "prevention" of the development of spontaneous hypertension by repeated denervations of the kidney [13].

In ischemic renal hypertension both a reduction in the number of intrarenal nerves (the ischemic kidney in bilateral — one clip two kidney — hypertension) [3] and strengthening of the intrarenal innervation (the sole, also ischemic, kidney in unilateral — one clip one kidney — hypertension) [10] have been demonstrated in the ischemic kidney. Preservation of the innervation of the "intact" kidney, at first glance is not absolutely clear. Apparently to increase the tone of the afferent arterioles — an important element in "resetting" — strengthening of the intrarenal sympathetic innervation is necessary, but this was not observed. To maintain water and electrolyte homeostasis within normal limits, which is the task under these conditions mainly of the "intact" kidney, maintenance of the normal number of nerve elements in it is optimal. Differences in the intensity of innervation of the kidney in spontaneous and ischemic renal hypertension evidently reflect differences in their pathogenesis: predominantly nervous mechanisms of development in the first case and humoral in the second.

LITERATURE CITED

1. Yu. V. Postnov, K. A. Fofanova, and G. A. Fedina, Kardiologiya, No. 11, 15 (1970).
2. Yu. V. Postnov, Kardiologiya, No. 12, 30 (1979).
3. L. Barajas, P. Wang, C. M. Bennet, and R. L. Wilburn, Lab. Invest., 35, 574 (1976).
4. R. Dietz et al., Circulat. Res., 43, Suppl. 1, 98 (1978).
5. J. Eide, M. R. Myers, V. de Quattro, et al., J. Cardiovasc. Pharm., 2, 833 (1980).
6. L. L. Francisco, L. L. Sawin, and G. F. di Bona, Hypertension, 3, 134 (1981).
7. A. C. Guyton, T. I. Coleman, and H. J. Granger, Annu. Rev. Physiol., 34, 13 (1972).
8. W. V. Judy and S. D. Farrell, Hypertension, 1, 605 (1979).
9. M. Karnovsky and L. Roots, J. Histochem. Cytochem., 12, 219 (1964).
10. R. E. Katholi, Hypertension, 3, 404 (1981).
11. R. L. Kline, P. M. Kelton, and P. F. Mercer, Can. J. Physiol. Pharmacol., 56, 818 (1978).
12. J. F. Liard, Experienta, 33, 339 (1977).
13. R. A. Norman and D. J. Drielak, Am. J. Physiol., 243, H294 (1982).
14. P. Thoren and S.-E. Ricksten, Clin. Sci., 57, 197 (1979).
15. J. C. Torre and J. W. Surgeon, Histochemistry, 49, 81 (1976).
16. K. B. Touw, J. R. Haywood, R. A. Shaffer, and M. J. Brody, Hypertension, 2, 408 (1980).
17. S. R. Winternitz, R. E. Katholi, and S. Oparil, J. Clin, Invest., 66, 971 (1980).