ENZYME-HISTOCHEMICAL STUDY OF FUNCTION OF THE MEDULLA OBLONGATA IN RATS WITH RENOVASCULAR HYPERTENSION

G. É. Galust'yan

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Renovascular hypertension (RVH) is one of the most widely used models of arterial hypertension [3]. It is traditionally considered that the blood pressure (BP) rises under these conditions as a result of the action of humoral factors (angiotensin or sodium) on peripheral vascular tone [9]. However, we know that central desympathization or destruction in the anterior hypothalamus can prevent the development of RVH [6, 7], evidence that central mechanisms also are involved in the pathogenesis of the disease. An important role in the central regulation of the circulation is played by structures of the medulla oblongata [1, 2, 5]. One way of assessing function of different parts of the brain is to study the intensity of their energy metabolism. Investigations by autoradiography with labeled deoxyglucose have shown that the development of spontaneous hypertension in rats, and also acute fluctuations of BP, are accompanied by changes in metabolic activity of several structures of the medulla [11, 14]. No experiments of this kind have been conducted on animals with RVH.

In the investigation described below activity of the medullary vasomotor structures was studied in rats with RVH by estimating activity of enzymes of energy metabolism: succinate dehydrogenase (SDH; EC 1.3.99.1) and NADH-dehydrogenase (NADH-DH; EC 1.6.99.3).

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 160-220 g. Under general anesthesia (pentobarbital sodium, 40 mg/kg) both renal arteries were stenosed by application of Lavsan* ligature. The degree of stenosis was controlled by means of a chuck, 0.15 mm in diameter, which was applied to the artery and removed after the knot had been tied. Systolic BP in the caudal artery was measured periodically by a photoelectric method under superficial ether anesthesia. For subsequent work animals whose systolic BP was not below 170 mm Hg were chosen. The average time from the operation to removal of the experimental material was 150 ± 3 days. The hypertensive and control rats were decapitated and the medulla was isolated and frozen in iso-octane, cooled with liquid nitrogen. The region of the medulla at level P 7.0-P 8.0, according to the atlas [13], was investigated. SDH and NADH-DH activity was demonstrated in sections 10 μ thick, cut on a freezing microtome, by the usual histochemical methods [4]. Enzyme activity was determined on the MTsFU-2 photometer-microscope by the "plug" method. The results of the measurements were subjected to statistical analysis on the Élektronika D3-28 computer.

EXPERIMENTAL RESULTS

The systolic BP in rats with RVH, measured by the indirect method, was 179 ± 4 mm Hg ($n = 8$) compared with 108 ± 3 mm Hg in the control animals ($n = 7$, $P < 0.001$). At autopsy, atrophy of one kidney was found in 87.5% of the hypertensive rats.

A significant increase in STH activity was observed in the ventral reticular and commissural nuclei of the rats with RVH and a decrease in neurons of the dorsal nucleus of the vagus nerve and n. ambiguus. Significant changes in NADH-DH activity were found only in the nucleus of the hypoglossal nerve: a decrease in the neurons and an increase in the neuropil (Table 1).

*Equivalent of Dacron.

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TABLE 1. SDH and NADH-DH Activity in Medullary Structures of Rats with Renovascular Hypertension

Structure	Enzyme activity, optical density units of NADH-DH			
	SDH		NADH-DH	
	control	hypertension	control	hypertension
Nucleus of hypoglossal nerve:				
neurons	0,475±0,019	0,440±0,023	0,442±0,009	0,343±0,011‡
neuropil	0,337±0,018	0,359±0,017	0,238±0,010	0,280±0,013*
Dorsal nucleus of vagus nerve				
neurons	0,424±0,016	0,394±0,018	0,345±0,012	0,311±0,019
neuropil	0,326±0,017	0,283±0,058	0,221±0,007	0,214±0,016
Nucleus of tractus solitarius	0,325±0,010	0,289±0,019	0,247±0,007	0,258±0,016
Nucleus of spinal tract of trigeminal nerve	0,369±0,019	0,385±0,022	0,277±0,011	0,283±0,015
Nucleus gracilis	0,407±0,024	0,388±0,025	0,303±0,010	0,292±0,014
Nucleus cuneatus	0,439±0,026	0,451±0,016	0,295±0,011	0,303±0,011
Dorsal reticular nucleus	0,304±0,026	0,316±0,014	0,257±0,012	0,241±0,008
Ventral reticular nucleus	0,295±0,10	0,334±0,012*	0,260±0,008	0,259±0,011
Paramedian reticular nucleus	0,236±0,008	0,242±0,022	0,229±0,013	0,257±0,012
Lateral reticular nucleus	0,366±0,017	0,326±0,023	0,251±0,008	0,282±0,013
Parvocellular reticular nucleus	0,313±0,032	0,264±0,028	0,288±0,012	0,244±0,018
Nucleus intercalatus:				
neurons	0,384±0,039	0,345±0,019	0,328±0,10	0,321±0,012
neuropil	0,266±0,039	0,291±0,030	0,210±0,014	0,206±0,013
Commissural nucleus	0,243±0,025	0,314±0,008*	0,228±0,017	0,262±0,021
Nucleus ambiguus:				
neurons	0,475±0,017	0,334±0,027 †	0,339±0,019	0,357±0,027
neuropil	0,308±0,018	0,261±0,006	0,231±0,012	0,262±0,017
Inferior olivary nucleus	0,402±0,022	0,358±0,021	0,294±0,019	0,317±0,024

Legend. *P < 0.05, †P < 0.01, ‡P < 0.001, compared with control.

TABLE 2. Correlation Between Enzyme Activity in Medulla of Normotensive and Hypertensive Rats

Enzyme	Coefficient of correlation (r)	t_0	P	Coefficient of regression (b)	t_1	P
SDH	0,762	4,5603	<0,001	0,589	2,8725	<0,05
NADH-DH	0,822	5,7752	<0,001	0,536	4,7162	<0,001

Changes also were observed in enzyme activity in various other structures, although these were not significant. Regression analysis revealed strong correlation between enzyme activity in the brain structures of the control and hypertensive animals (Fig. 1; Table 2). A general tendency was observed for the maximal values of activity to fall and the minimal values to rise. On the whole, hypertension had a significant general effect on SDH and NADH-DH activity in the medullary structures, and this was confirmed by the significance of the difference of the linear regression coefficients from unity (Table 2).

The pathogenesis of hypertension of the "two kidney – two clips" and "one kidney – one clip" type is similar and is associated primarily with retention of sodium and water, and not with secretion of angiotensin [10]. Accordingly, gradual atrophy of one kidney in the present experiments probably did not give rise to significant changes in the time course of development of hypertension.

An increase in the contents of adrenalin and noradrenalin and activation of their metabolism have been found in the medulla of rats with RVH and the "two kidneys – two clips" and "one kidney – one clip" types [10]. These changes were interpreted by the authors cited as activation of both pressor and depressor mechanisms. As will be clear from Table 1, the greatest changes in metabolic activity in RVH were observed mainly in structures participating in regulation of the circulation [2, 5]. On the whole, however, these changes were rather diffuse in character. It is difficult at present to decide which processes are reflected in these changes in medullary energy metabolism. One such process is probably the direct response of central vasomotor structures to sodium and water retention, connected with a decrease in renal filtration. At the same time, a diffuse and less specific action on nerve tissue is possible, connected with humoral control mechanisms, for example, through a change in the ion transport system. Data showing a decrease in Na,K-ATPase activity in the myocardium and vessels in volume-dependent RVH [8, 12] indicate that this is a valid suggestion.

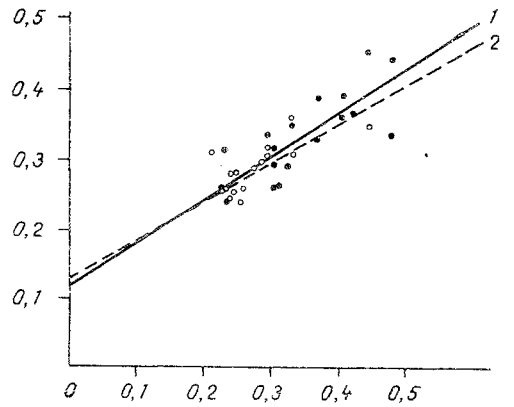

Fig. 1. Correlation between dehydrogenase activity in medullary structures of normotensive rats and rats with RVH. Horizontal axis, enzyme activity (in optical density units) in normotensive rats; vertical axis, the same in rats with RVH. 1) SDH, 2) NADH-DH.

Whatever their mechanisms may be, the changes observed in brain energy metabolism are evidence of the existence of a central component of the pathogenesis of renal hypertension.

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