PHYSIOLOGY

Functional Activity of the Sympathoadrenal System in Hypertensive NISAG Rats

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NISAG rats with stress-induced arterial hypertension are characterized by hyperactivity of the sympathoadrenal system under rest conditions and during stress exposure.

Key Words: *arterial hypertension; sympathoadrenal system; stress*

NISAG rats were selected by hyperresponse of blood pressure to emotional stress (30-min restriction in a cylindrical wire cage) [7]. Systolic blood pressure in animals of this strain is elevated under rest conditions (170 mm Hg) and sharply increases upon stress exposure (up to 200 mm Hg) [8]. NISAG rats are characterized by functional hyperfunction of the major neurohormonal stress system — the hypothalamic-pituitary-adrenocortical system [3]. Previous studies showed that activity of the sympathoadrenal system increases under certain conditions [7,9]. Here we studied functional activity of the sympathoadrenal system in NISAG rats.

MATERIALS AND METHODS

Experiments were performed on normotensive male WAG rats and hypertensive NISAG rats aging 5-6 months. They were maintained at the Laboratory of Experimental Animals (Institute of Cytology and Genetics) under standard conditions. The animals received a well-balanced food and water *ad libitum*. Experimental procedures were conducted according to the requirements of the International Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

The animals were divided into 3 groups. Group 1 rats of both strains were decapitated. The adrenal glands were isolated, immediately frozen in liquid nitrogen, and stored until catecholamine assay. The adrenal glands from group 2 rats were used to estimate the level of tyrosine hydroxylase gene mRNA. Some animals of group 2 were exposed to 2-h restriction in cylindrical cages to study induction of this gene under stress conditions. These rats were killed 2 h after the end of the stress exposure. A catheter was implanted into the common carotid artery of group 3 animals to measure catecholamine concentration in blood samples. Implantation was performed under nembutal anesthesia. Blood samples were obtained from awake animals over the next 3 days.

Blood pressure was measured indirectly in the caudal artery. The concentration of catecholamines (epinephrine, norepinephrine, and dopamine) in the adrenal glands and blood plasma was measured by high-performance liquid chromatography with electrochemical detection. Expression of the gene encoding the key enzyme of catecholamine biosyn-

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thesis (tyrosine hydroxylase) in the adrenal glands was studied by reverse transcription polymerase chain reaction (PCR) in a real-time mode. Total RNA was isolated by the phenol method. The reverse transcription reaction with MOMLV polymerase was performed to synthesize cDNA. The tyrosine hydroxylase gene mRNA level (*TH*) was determined with a system containing AmpliTaq-Gold DNA polymerase, primers specific for tyrosine hydroxylase genes and ribosomal RPL29 protein (internal standard), and FAM-labeled sequence-specific probes (Applied Biosystems). PCR was conducted in 20 μl buffer mixture as follows: 94°C, 10 min; 94°C, 30 sec; and 72°C, 2 min (40 cycles). Real-time detection of the end product was performed using sequence-specific probes (TaqMan) carrying a terminal fluorescent agent and fluorescence quencher. Synthesis of a new chain with 5'-3'-exonuclease results in fluorochrome detachment, probe degradation and, therefore, increase in fluorescence of the solution. Nonspecific products of synthesis were undetected under these conditions, which allowed us to estimate mRNA level.

The results were analyzed by Student's *t* test for small samples.

RESULTS

Epinephrine is the major catecholamine in the adrenal glands in rats (Table 1). The concentration of epinephrine is one order of magnitude higher than that of norepinephrine and dopamine. Significant interstrain differences were revealed. Epinephrine concentration in NISAG rats was 2-fold higher than in WAG rats. The concentrations of norepinephrine and dopamine in WAG rats were higher than in NISAG rats. These differences are probably related to rapid conversion of intermediate products into end product epinephrine in NISAG rats with high rate of biosynthetic processes.

TABLE 1. Concentration of Norepinephrine, Epinephrine, and Dopamine in the Adrenal Glands of WAG and NISAG Rats ($X \pm m$, n=10)

Biogenic amine	NISAG	WAG
Norepinephrine, ng	9152±859	17,018±1071**
Epinephrine, ng	11,3533±6410	69,163±5632**
Dopamine, ng	430±53	728±103*

Note. *p<0.05 and **p<0.001 compared to NISAG rats.

The sympathoadrenal response of rats to emotional stress was studied in experiments with chronic artery cannulation. The blood was taken from awake rats over 3 days. The animals were immobilized for a short time (3 min). Probably, the first sampling of blood was associated with the state of emotional stress. The severity of stress under these conditions exceeded that observed in repeated sampling of blood (Table 2). On day 1 norepinephrine concentration in NISAG rats was 2 times higher than in WAG rats. On day 2 the mean concentration of norepinephrine in the plasma remained high in NISAG rats, but no interstrain differences were revealed in this period. Norepinephrine concentration in NISAG rats on day 3 was much lower than on day 1. During this period norepinephrine concentration in NISAG rats was lower than in WAG rats. It should be emphasized that norepinephrine concentration in WAG rats remained practically unchanged over 3 days. In WAG rats epinephrine concentration on day 1 was 2-fold higher than on day 3. However, in NISAG rats epinephrine concentration on day 1 was one order of magnitude higher than on day 3. On days 1 and 2 epinephrine concentration in NISAG rats was higher than in

TABLE 2. Concentration of Norepinephrine, Epinephrine, and Dopamine in Peripheral Blood Samples Taken through a Chronically Implanted Arterial Catheter over 3 Days ($X \pm m$, n=10)

Biogenic amine	Days of sampling	WAG (n)	NISAG (n)
Norepinephrine, pg/ml	1	241±38 (12)	550±109 (19)*
	2	298±73 (11)	604±141 (15)
	3	299±38 (10)	155±22 (12)**$^{++}$
Epinephrine, pg/ml	1	239±49 (12)	672±165 (17)*
	2	238±53 (11)	650±121 (14)**
	3	104±32 (8)$^{+}$	30.5±7.7 (10)*$^{+++}$
Dopamine, pg/ml	1	93±21 (12)	88±19 (15)
	2	131±31 (7)	74±14 (13)
	3	103±14 (8)	76±13 (9)

Note. *p<0.05 and **p<0.01 compared to WAG rats; ^{+}p<0.05, ^{++}p<0.01, and ^{+++}p<0.001 compared to day 1.

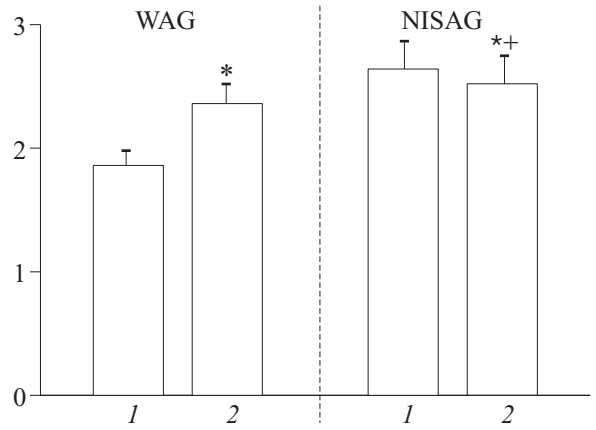

Fig. 1. Tyrosine hydroxylase gene mRNA level (arb. units) in the adrenal glands of Wistar (WAG) and NISAG rats under rest conditions (*1*) and 2 h after 2-h restriction stress (*2*). $p<0.05$: *compared to the control; +compared to WAG rats.

WAG rats. On day 3 epinephrine concentration in WAG rats was higher than in NISAG rats. Plasma dopamine concentration in animals of both strains remained practically unchanged in various periods.

Under rest conditions the level of tyrosine hydroxylase gene mRNA in NISAG rats was much higher than in WAG rats (Fig. 1). However, restriction stress led to additional induction of this gene only in WAG rats. The level of tyrosine hydroxylase gene mRNA in WAG rats after restriction stress did not differ from that observed in NISAG rats under rest conditions. In NISAG rats restriction stress was not accompanied by further increase in the expression of tyrosine hydroxylase gene in the adrenal glands. It can be hypothesized that expression of tyrosine hydroxylase gene in NISAG rats is permanently high and corresponds to transcriptional activity of this gene in normotensive animals under stress conditions.

Stress-induced hypertension in NISAG rats is probably related to their increased stress reactivity. It results from hyperfunction of the pituitary-adrenocortical and sympathoadrenal systems. Blood pressure in NISAG rats sharply increases during emotional stress, which is probably associated with stimulation of epinephrine secretion in the adrenal medulla and increased release of norepinephrine from sympathetic nerve endings. The concentration of these amines in the plasma significantly increases during stress. High level of blood pressure in NISAG rats under rest conditions is related to the influence of other factors, since the concentrations of epinephrine and norepinephrine in the plasma of adapted animals do not differ from normal. This specific feature can be associated with increased sensitivity of effector systems to vasoconstrictor influences, which was previously demonstrated in *in vitro* experiments [1]. Other neurohormonal mechanisms cannot be excluded (*e.g.*, increase in thyroid activity [6], adrenocortical hyperfunction, and potentiation of vasoconstriction by glucocorticoid hormones). The increase in basal blood pressure can result from morphological changes in the target organs of NISAG rats [2,4,5]. The frequency of episodes of blood pressure elevation increases due to hyperreactivity of the sympathoadrenal system in NISAG rats, which contributes to progression of these changes. Much recent attention is focused on the renal mechanisms of blood pressure regulation and function of the renin-angiotensin-aldosterone system in NISAG rats.

Strong evidence exists that the pathogenesis of arterial hypertension involves increased sympathetic activity [10]. Our experiments on the model of stress-induced arterial hypertension showed that this form of arterial hypertension is accompanied by significant functional activation of the sympathoadrenal system. These changes probably determine a specific neurohormonal background inducing activation of various peripheral and neurohormonal mechanisms leading to permanent increase in blood pressure in the absence of stress factors.

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