# Norepinephrine Content in the Hypothalamus and Medulla Oblongata of ISIAH Rats is Regulated by Several Genetic Loci

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Five genetic loci regulating epinephrine content in rat hypothalamus and two loci in the medulla oblongata were identified using polymorphic microsatellite DNA markers. Allele polymorphism of these loci determines the differences between norepinephrine levels in brain compartments of hypertensive ISIAH and normotensive WAG rats. The interactions between the detected epinephrine-regulating loci are additive.

**Key Words:** *brain norepinephrine; genetic regulation; hypertensive ISIAH rats*

Study of the neuroendocrine reactions is a leading trend in research of common and specific problems in the pathogenesis of stress. Activation of the sympathoadrenal system in response to stress factors is an important stage of adaptation. At the same time, it can serve as a mechanism of disease formation ("adaptation disease"), including formation of essential hypertension [2]. ISIAH rats (stress-induced hereditary hypertension) are characterized, in addition to high blood pressure, by high activity of the sympathoadrenal system [1,5] and serve as an adequate model for the search for genetic factors responsible for the formation of stress-dependent hypertensive status. Differences in catecholamine levels in different brain compartments of normo- and hypertensive rats can determine specific features of hypertensive status formation [4], but genetic determination of the cerebral noradrenergic system in hypertensive rats is studied insufficiently.

We attempted the search for genetic loci regulating the differences in norepinephrine levels in the hypothalamus and medulla oblongata of hypertensive ISIAH and normotensive WAG rats.

## MATERIALS AND METHODS

The study was carried out on male rats with inherited stress-induced arterial hypertension (ISIAH) and normotensive Wistar Albino Glaxo (WAG) rats. The search for loci regulating norepinephrine level in the hypothalamus and medulla oblongata was carried out using microsatellite DNA markers in genetically segregating population of 6-month male (ISIAH×WAG) $F_2$ hybrids. The rats ($n$=126) were kept under standard vivarium conditions with free access to water and food. Experiments were carried out in accordance with the international standards for manipulations on laboratory animals.

For measuring norepinephrine levels, the rats were rapidly decapitated, the hypothalamus and medulla oblongata were removed and stored at -80°C until the assay. Norepinephrine concentrations were measured by the microspectrofluorometric method on an MPF-4 microspectrofluorometer (Hitachi) [8].

Microsatellite analysis was carried out with DNA isolated from the liver using proteinase K and phenol extraction by the standard method [7]. Then DNA

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was re-precipitated and dissolved in deionized water. Microsatellite markers were selected from databases http://www-genome.wi.mit.edu and http://www.well.ox.ac.uk. The position of the markers on chromosomes expressed in millions of nucleotides (megabases; Mb) was estimated by the rat genome sequence data (http://www.ensembl.org/Rattus_norvegicus/). The markers were selected evenly along the entire length of chromosomes at a distance of 15-20 Mb. Analysis of DNA of male $F_2$ hybrid population was carried out with 147 polymorphic markers. Primers of the following markers were used in this study: D4Rat27 (71.4 Mb), D4Rat68 (172.17 Mb), D14Rat18 (85.72 Mb), D18Rat106 (14.05 Mb), DXRat26 (63.2 Mb), D1Rat54 (168.0 Mb), D3Rat27 (87.9 Mb). Primer annealing temperature varied from 60 to 64°C. The PCR on the rat genome DNA was carried out as described previously [6].

The results were statistically processed using Statistica 6.0 software, the significance of differences between the means was evaluated using Student's *t* test. Associations of DNA markers with norepinephrine concentrations in brain structures were evaluated by one-way ANOVA.

## RESULTS

The mean concentrations of norepinephrine in the hypothalamus (1.93±0.07 μg/g) and medulla oblongata (0.25±0.01 μg/g) were measured in male $F_2$ hybrids. Using microsatellite DNA analysis, 5 loci regulating norepinephrine level in the hypothalamus and 2 loci for the medulla oblongata were detected. Loci with the elevating and reducing effects on norepinephrine level in brain structures were found (Table 1). The decrease in norepinephrine level in the hypothalamus of ISIAH rat was associated with markers D4Rat27 and D4Rat68, its increase with markers D18Rat106 and DXRat26. The reduction of norepinephrine level in the medulla oblongata was associated with D1Rat54 marker, its elevation with D3Rat27 marker.

Predominance of ISIAH rat alleles was observed in chromosome 4 labeled loci. Norepinephrine level in the hypothalamus of D14Rat18 marker heterozygotes was significantly higher than in allele homozygotic WAG and ISIAH rats. This indicates the heterosis effect in this locus. Co-predominance of alleles was detected in the D18Rat106 marker locus. These data suggest an intricate genetic regulation of norepinephrine level in rat hypothalamus. The ISIAH rat allele in D1Rat54 marker locus regulating norepinephrine level in the medulla oblongata is dominant, while in the D3Rat27 locus it is recessive. The dominant alleles reduce norepinephrine level in the medulla oblongata.

**TABLE 1.** Impact of ISIAH and WAG Rat Alleles for Norepinephrine Levels in Brain Structures

| Brain compartment; chromosome | | Marker | I/I | | I/W | | W/W | | Effect of ISIAH rat alleles | *P* (ANOVA) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *M±m* | *n* | *M±m* | *n* | *M±m* | *n* | | |
| Norepinephrine concentration in hypothalamus, μg/g | 4 | D4Rat27 | 1.80±0.11* | 31 | 1.790±0.076 | 56 | 2.27±0.16 | 36 | -0.47 | 0.0046 |
| | 4 | D4Rat68 | 1.87±0.13* | 25 | 1.800±0.069 | 74 | 2.39±0.21 | 24 | -0.52 | 0.0027 |
| | 14 | D14Rat18 | 1.72±0.085$^{+\dagger}$ | 30 | 2.14±0.12 | 58 | 1.760±0.088$^{+}$ | 35 | -0.04 | 0.0095 |
| | 18 | D18Rat106 | 2.21±0.15** | 29 | 1.970±0.096 | 58 | 1.65±0.11 | 38 | +0.56 | 0.0064 |
| | X | DXRat26 | 2.17±0.13** | 52 | | | 1.75±0.06 | 71 | +0.42 | 0.0017 |
| Norepinephrine concentration in medulla oblongata, μg/g | 1 | D1Rat54 | 0.23±0.02* | 26 | 0.220±0.012 | 66 | 0.32±0.04 | 34 | -0.09 | 0.0043 |
| | 3 | D3Rat27 | 0.33±0.05 | 27 | 0.230±0.013 | 61 | 0.23±0.02 | 38 | +0.10 | 0.0082 |

**Note.** *$p<0.05$, **$p<0.01$ compared to rats homozygotic by WAG allele; $^{+}p<0.05$, $^{++}p<0.01$ compared to heterozygote. Here and in Tables 2, 3: I/I: ISIAH allele homozygotes; W/W: WAG allele homozygotes.

**TABLE 2.** Effects of Interactions between Loci Regulating Norepinephrine Level in the Hypothalamus

| Marker | D4Rat27 | D4Rat68 | D18Rat106 | DXRat26 | Norepinephrine concentration, μg/g | | Impact of ISIAH alleles |
|---|---|---|---|---|---|---|---|
| | | | | | *M*±*m* | *n* | |
| Genotype | I/I | I/I | | | 1.84±0.21 | 11 | -0.63 |
| | W/W | W/W | | | 2.47±0.36 | 13 | |
| | | | I/I | I/I | 2.70±0.23** | 14 | +1.04 |
| | | | W/W | W/W | 1.66±0.15 | 22 | |
| | I/I | I/I | W/W | W/W | 1.26±0.37 | 2 | 2.12[+] |
| | W/W | W/W | I/I | I/I | 3.38±0.62* | 3 | |

**Note.** *$p$<0.05, **$p$<0.001 compared to homozygotic WAG rats. [+]Effect of 4 loci alleles.

The presence of several loci with different types of allele interactions in the rat genotype determined significant combination variability of the sign. The impact of the detected loci for norepinephrine level in the hypothalamus in cases with co-inheriting of two or four attenuating and enhancing loci is presented in Table 2. The data indicate additive locus-locus interactions. Evaluation of the combined effects of loci on chromosomes 18 and X clearly showed the additive effect of the loci: the difference between ISIAH and WAG rat allele double homozygotes was +1.04 (Table 2), which was virtually equal to the sum of individual effects of homozygotes by these loci (0.56+0.42=0.98; Table 1). For combination D4Rat27 and D4Rat68 marker locus, the difference in the hypothalamic norepinephrine level was -0.63 (Table 2), while the sum of individual effects of each of these loci was -0.99 (Table 1). This deviation from the additive effect can be explained by the predominance in this group of animals with D18Rat106 and DXRat26 marker loci WAG alleles, reducing norepinephrine level in the hypothalamus. Analysis of genetic loci modulating (elevating or reducing) norepinephrine level in the medulla oblongata also suggests the additive nature of locus-locus interactions (Table 3).

Previous mapping of loci involved in the reaction of the sympathetic nervous system to stress in $F_2$ male hybrids (hHTg×BN) detected 2 loci on chromosome 10 regulating plasma norepinephrine level [3]. Our findings indicate that the differences in norepinephrine levels in the hypothalamus and medulla oblongata in hyper- and normotensive rats are associated with polymorphism of several genetic loci, some of them leading to elevation of norepinephrine concentration in the hypothalamus and medulla oblongata, others to its reduction. The effect of these changes seems to depend on specific function of the brain structure in which these changes occur. The results of the study suggest specific genetic regulation of norepinephrine levels for different tissues in rats of different strains. Many-year selection of ISIAH rats by the maximum elevation of blood pressure under the effect of mental stress led to fixation of specific genetic changes in norepinephrine metabolism.

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**TABLE 3.** Effect of Interactions between Loci Regulating Norepinephrine Level in the Medulla Oblongata

| Marker | D1Rat54 | D3Rat27 | Norepinephrine concentration, μg/g | | Impact of ISIAH alleles |
|---|---|---|---|---|---|
| | | | *M*±*m* | *n* | |
| Genotype | I/I | W/W | 0.24±0.03 | 7 | -0.19 |
| | W/W | I/I | 0.43±0.11 | 11 | |
| | I/I | I/I | 0.26±0.05 | 5 | -0.04 |
| | W/W | W/W | 0.30±0.04 | 10 | |

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