

## Experimental Research

## Study of Hypertension in Spontaneous Hypertensive Rats by Sequencing the Genomic DNA of Alpha<sub>2B</sub> Receptors

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Key words:

**Sequencing genomic DNA, SHR rats, alpha<sub>2B</sub> adrenergic receptors.**

**Introduction:** The origin of hypertension in the genetic model of spontaneous hypertensive rats (SHR) is still unknown. Since the sympathetic nervous system (SNS) plays an important role in this experimental model, we attempted to explore the possible pathophysiological mechanisms of this hypertension by focusing on potential mutations of the alpha<sub>2B</sub> receptors of the SNS.

**Materials and Methods:** We studied the nucleotide sequencing of genomic DNA from 8 rats, 4 SHR, 2 Wistar-Kyoto, 1 Dahl-S and 1 Dahl-R and compared the results with the published sequencing of alpha<sub>2B</sub> adrenergic receptors in the National Center for Biotechnology Information (NCBI), as well as against each other.

**Results:** There were no important differences between all the above rats in the 6,268 nucleotides of the alpha<sub>2B</sub> receptor.

**Conclusion:** Our data suggest that, although the alpha<sub>2B</sub> adrenergic receptor has a crucial role in the development of hypertension, the genetic susceptibility or resistance of various rat strains to hypertension cannot be attributed to genetic polymorphisms in this receptor's gene.

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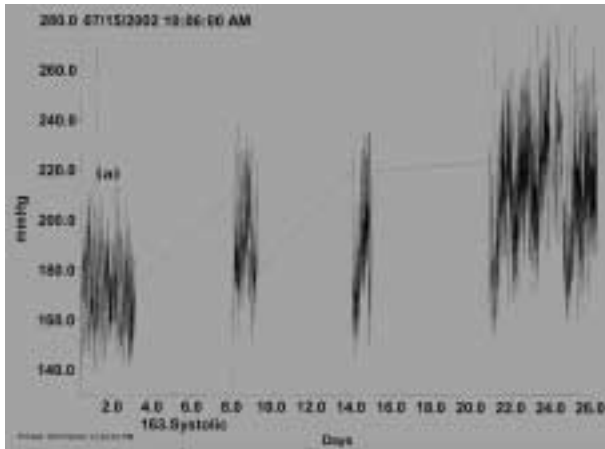
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**S**pontaneous hypertensive rats (SHR) are a genetic model of hypertension that is widely accepted in medical research because of the features they share with idiopathic hypertension in humans<sup>1</sup>.

During the last four decades, many researchers have attempted to determine the aetiology of hypertension in SHR by comparing this family of hypertensive experimental animals with other types of hypertensive (Dahl salt-sensitive) or normotensive (Wistar-Kyoto, Dahl salt-resistant) rats. The results have clearly demonstrated differences between SHR and normotensive rats with regard to the probable pathophysiological mechanisms implicated in hypertension, with special emphasis on the sympathetic nervous system.

Since hypertension is often accompanied by a state of over-stimulation of the sympathetic nervous system<sup>2</sup>, it has been postulated that there is dysfunction of a central adrenergic mechanism in SHR, leading to an increase in noradrenaline release and subsequent hypertension<sup>3</sup>. Indeed, it has been proven that various adrenergic mechanisms show alterations in genetic models of hypertension, including SHR<sup>4</sup>.

The sympathetic nervous system and its receptors are known to play an active part in the pathogenesis of idiopathic hypertension. There are three known subtypes of the alpha<sub>2</sub> adrenergic receptors (alpha<sub>2A</sub>, alpha<sub>2B</sub>, alpha<sub>2C</sub>). The alpha adrenergic receptors of the sympathetic nervous system are divided into two major types alpha<sub>1</sub> and alpha<sub>2</sub>, each of which



**Figure 1.** Graphical representation of the intra-aortic monitoring of fluctuations in systolic blood pressure in the SHR experimental animals that underwent genetic analysis. The figure clearly shows an initial value of 170 mmHg in one of the rats studied that remained steady over a three-day period (a) with no intervention.

contains three subtypes. Subtype  $\alpha_{2A}$  leads to suppression of the sympathetic nervous system and ensuing hypotension, whereas subtype  $\alpha_{2B}$  stimulates sympathetic activity and causes a hypertensive response. Indeed, genetically engineered mice with deleted  $\alpha_{2B}$  adrenergic receptor genes are incapable of increasing their blood pressure even under acute or chronic NaCl loading<sup>6,7</sup>.

Since the  $\alpha_{2B}$  adrenergic receptors mediate the hypertensive response to salt loading, we hypothesized that the presence of one or more point mutations on their genome, compared with the genes of other strains of hypertensive or normotensive rats, might result in their over-expression and could thus be one of the mechanisms responsible for triggering and sustaining hypertension in SHR.

Genomic DNA sequencing provides us with a way to investigate this hypothesis in the context of a study of the pathophysiology of hypertension in SHR.

## Material and Methods

Our study focused on the analysis of nucleotide sequencing of the genome of the  $\alpha_{2B}$  adrenergic receptor in different strains of rats. The study group consisted of 4 hypertensive inbred SHR (Figure 1), while the control group was made up of 4 normotensive inbred rats (2 Wistar-Kyoto, 1 Dahl-S and 1 Dahl-R).

The study protocol included the following stages and was based on previously described techniques<sup>8</sup>:

- Extraction of genomic DNA from the blood of each rat;
- Isolation of the gene of the  $\alpha_{2B}$  adrenergic receptor with design of suitable primers for each genomic region;
- Quantitative analysis of the isolated genome using the polymerase chain reaction (PCR) method;
- Analysis of the nucleotide sequencing of the genome of the  $\alpha_{2B}$  adrenergic receptor, which contains 6,268 nucleotide bases;
- Comparison of the sequencing from each animal against each other and against corresponding data stored in the NCBI data bank (Figure 2).<sup>8</sup>

Briefly, 4 hypertensive SHR, 2 normotensive Wistar-Kyoto rats, 1 normotensive Dahl-S and 1 normotensive Dahl-R rat, all aged 18-20 weeks and weighing 320-350g, were anaesthetised with pentobarbital (50 mg/kg intra-peritoneal) and 3 ml blood was taken from the descending aorta of each animal. In the SHR the presence of hypertension had been verified earlier by the intra-arterial recording of a systolic blood pressure ranging from 160-180 mmHg. Genomic DNA was isolated from each blood sample using the following steps: lysis of red blood cells, lysis of the white blood cells (source of genomic DNA), addition of RNAase to destroy the RNA (only genomic DNA was to be isolated), precipitation of proteins, collection of fine fibrils of genomic DNA, and finally, measurement of the concentration of genomic DNA with the aid of a Beckmen spectrophotometer.

Along with the isolation of the genomic DNA, suitable primers were designed for the subsequent isolation of the  $\alpha_{2B}$  adrenergic receptor gene exclusively from the total quantity of genomic DNA (Figure 3a). Our source of information was an analysis of nucleotide sequencing already submitted to the NCBI data bank, which has locus AF366899 and refers to *Rattus Norvegicus*<sup>9</sup>. Subsequently:

- The gene of the  $\alpha_{2B}$  adrenergic receptor was isolated segmentally with the aid of the primers and enhanced by PCR, after the PCR solution had been prepared using Platinum Taq DNA Polymerase (Gibco) and dNTP set (Invitrogen).
- The presence of DNA was verified using electrophoresis (0.8% agarose gel) and ultraviolet radiation, after which (Figure 3b)
- the product was purified using a Qiaquick Multiwell PCR Purification Kit (Qiagen),
- the solution was prepared for the nucleotide sequencing reaction, using a DNA Sequencing Kit (Applied Biosystems) and PCR method,

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241  tgaatgctct  taagacctgt  gtaacagttc  gacctcgag  gggcagctga  atctggccgt
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6241  taggctgccc  tagaactcac  agagatct

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**Figure 2.** The information already submitted to the NCBI data bank with nucleotide sequences from the genome of the  $\alpha_{2B}$  adrenergic receptor.

- the final product was cleaned with the aid of an ABI Big Dye Kit (Qiagen),
- the purified product was sent to the Gene Core genetic laboratory of Boston University for reading of the nucleotide sequencing and detection of any point mutations.

**Results**

The results of the analysis of the nucleotide sequencing of the  $\alpha_{2B}$  adrenergic receptor were returned in graphical form (Figure 4) and were compared against each other, as well as against the sequence already submitted to the NCBI<sup>9</sup> data bank for the detection of any point mutations, especially in the transcribed portion of the gene (CDS), using the chromas program (version 2.21).

Comparing the nucleotide sequences from the hypertensive SHR with those from the normotensive controls (Wistar-Kyoto, Dahl-S, Dahl-R) gave the following findings:

The nucleotide sequences from the SHR, Wistar-Kyoto, Dahl-S and Dahl-R rats agreed completely among themselves and differed from the already published sequence of Schaak in the following three positions:

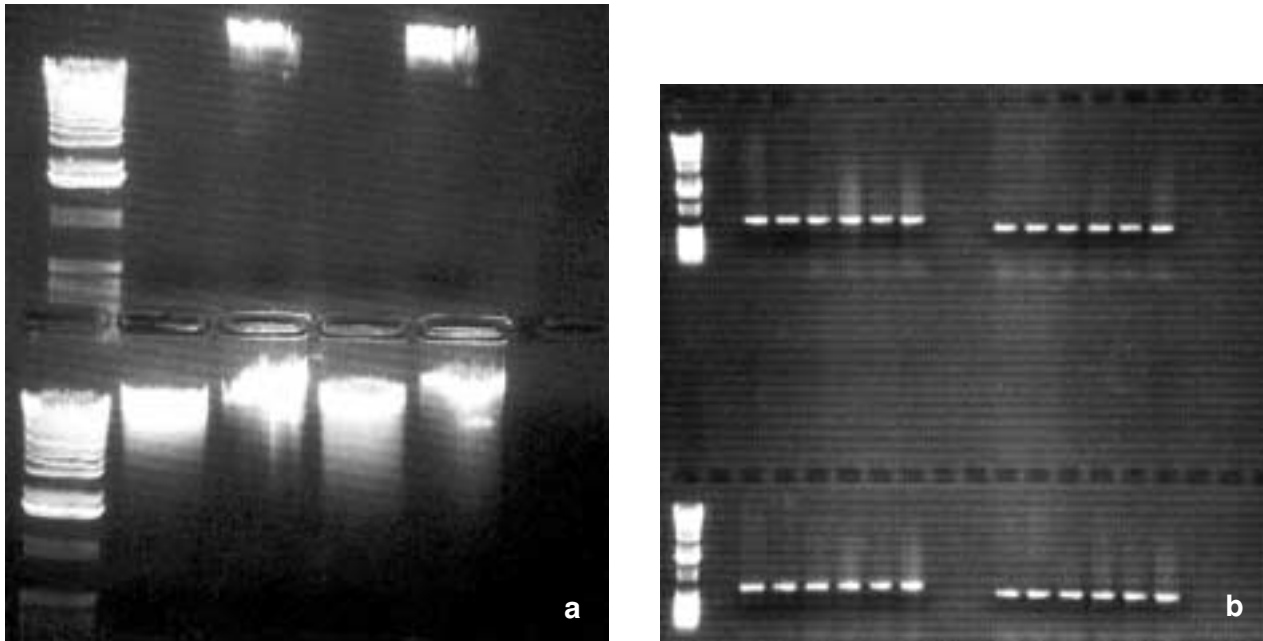
1. In position 5,653 cytosine (C) was found instead of adenine (A)
2. in position 5,655 cytosine (C) was found instead of thymine (T)
3. in position 5,672 cytosine (C) was found instead of thymine (T)

The above positions are from the mRNA region, but not in the CDS, and are not considered capable of affecting the gene’s functionality.

**Discussion**

SHR are considered to be a genetic model of hypertension analogous to idiopathic hypertension in humans. In spite of extensive research, the aetiology of hypertension in these rats remains unknown.

Many differences related to blood pressure have been reported in the literature between SHR and the normotensive Wistar-Kyoto rat<sup>10</sup>. An important difference is the degree of sympathetic activity, which appears to be particularly elevated in SHR, even before the appearance of the hypertension<sup>11,12</sup>. This finding is reinforced by the fact that bilateral sympathectomy in combination with removal of the ad-



**Figure 3 a, b.** Electrophoresis in 0.8% agarose gel of genomic DNA: six of the animals used in the study (Figure 3a) and part of the promoter region of the  $\alpha_{2B}$  adrenergic receptor gene of the same animals (Figure 3b). The 1Kb (250-10,000 bases) DNA ladder used each time for estimation of the size of the DNA can be clearly seen. The genomic DNA has a size of over 10,000 bases, while the section of the promoter region has around 1,000 bases.

renal medulla at a young age prevents the development of hypertension in SHR<sup>13-15</sup>.

It was precisely this revealing relationship between SHR hypertension and hyperactivity of the sympathetic nervous system that this study aimed to investigate. The study was at the genetic level and was designed to identify any point mutations, focusing on one of the genes involved in blood pressure control: the gene of the  $\alpha_{2B}$  adrenergic receptor. The gene of the  $\alpha_{2B}$  adrenergic receptor is a small-sized gene that appears only with a transcribed region, through which the protein of the  $\alpha_{2B}$  receptor of the cellular membrane is produced. The total size of this gene is 6,268 nucleotide bases and it is made up of the promoter region (nucleotide bases 1-2,407), the mRNA (nucleotide bases 2,408-5,989), and the polyA region (nucleotide bases 5,989-6,268). Within the mRNA region is the CDS region (nucleotide bases 2,780-4,141), which basically represents the functional region of the gene that is transcribed into mRNA and encoded in the protein receptor of the  $\alpha_{2B}$  cellular membrane<sup>9</sup>.

The current study did not find any significant point mutation at any position in the nucleotide sequence of the gene of the  $\alpha_{2B}$  adrenergic receptor in SHR compared with the nucleotide sequences of the

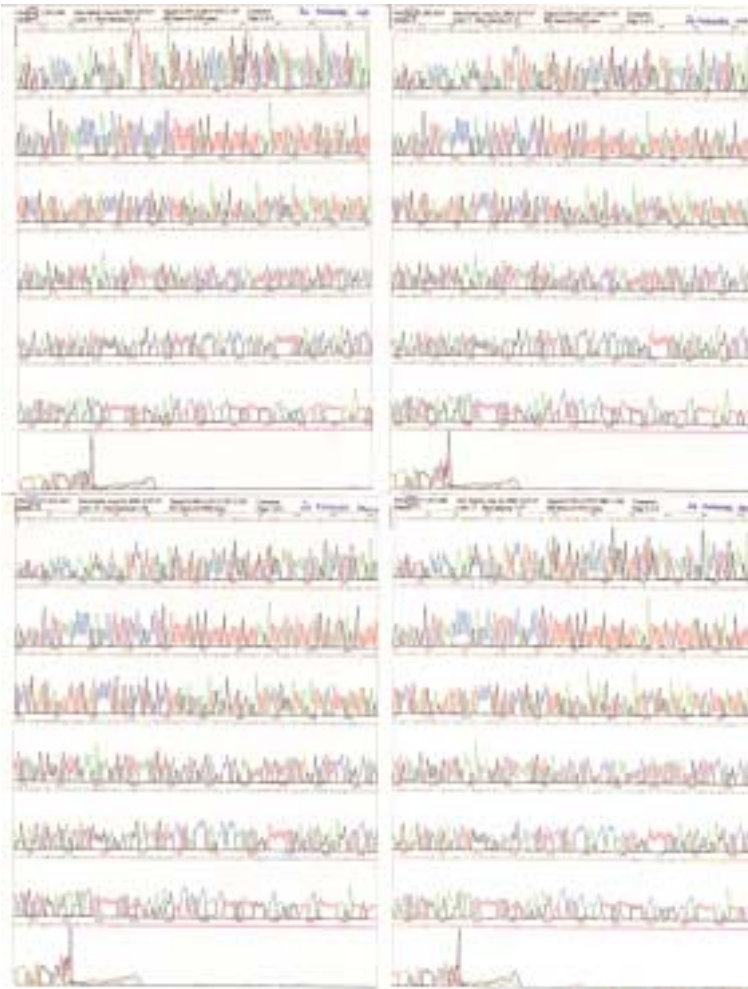
animals in the control group or with the previously known sequence in the NCBI data bank.

The adrenergic receptors of the sympathetic nervous system are divided into two major categories,  $\alpha_1$  and  $\alpha_2$ , each of which contains three subgroups. Subgroup  $\alpha_{2A}$  leads to suppression of the sympathetic nervous system and ensuing hypotension, whereas subgroup  $\alpha_{2B}$  stimulates sympathetic activity and causes a hypertensive response. Indeed, rats lacking the  $\alpha_{2B}$  gene pair are incapable of increasing their blood pressure even under acute or chronic NaCl loading<sup>6,7</sup>.

The  $\alpha_{2B}$  receptors, via the inhibitory Gi protein, inhibit the action of adenocyclase, resulting in a reduction in levels of cyclic AMP<sup>15</sup>. Whether these pathways are changed in SHR has not yet been determined.

The ventrolateral medulla is an important control position for blood pressure via the sympathetic nervous system, with main contributors the nucleus tractus solitari (NTS), the dorsal motor nucleus of the vagus nerve and the nucleus reticularis lateralis<sup>17</sup>.

Research findings have lead to the conclusion that experimental hypertension in rats is accompanied by changes in the ratio between the  $\alpha_1/\alpha_2$  adrenergic receptors in those regions of the brain associated



**Figure 4.** Graphical representations showing the nucleotide sequences from a section of the promoter region of the  $\alpha_{2B}$  adrenergic receptor gene following genetic analysis in SHR (s), Wistar-Kyoto (w), Dahl-S (ds) and Dahl-R (dr) rats. The initials represent nucleotide bases as follows: T-thymine, A-adenine, C-cytosine, G-guanine.

with the increase or decrease in blood pressure<sup>18</sup>. In addition, the brain stem sites where the  $\alpha_{2B}$  receptors are concentrated in SHR show a clear reduction in density<sup>19,20</sup>. Olmos et al<sup>21</sup> reached similar conclusions, finding a reduced density and sensitivity of  $\alpha_{2B}$  receptors in the brain of SHR.

Nomura et al<sup>22</sup>, finding that the connection points of  $\alpha_{2B}$  receptors to the NTS in the brain of SHR were already reduced by the age of four weeks, that is, before the onset of hypertension, hypothesised that these findings might be indicative of a genetic aetiology.

Accordingly, it appears that the  $\alpha_2$  receptors exhibit a clear qualitative and quantitative deficit in SHR compared with normotensive Wistar-Kyoto rats. Whatever this difference may be, our current study indicates that it is not due to a genetic variant of the  $\alpha_{2B}$  adrenergic receptors. Our findings are consistent with those of another study, which failed

to find genetic differences in the renal  $\alpha_{2B}$  adrenergic receptors genes between Sabra salt-sensitive versus Sabra salt-resistant rats.<sup>23</sup>

### Conclusions

The development of hypertension in SHR does not appear to be due to a genetic variation in the  $\alpha_{2B}$  adrenergic receptors, since analysis of the nucleotide sequence on their genome gives identical results in all strains of rats studied, regardless of whether they were susceptible or resistant to salt-dependent hypertension. Since, however, there is a considerable body of evidence implicating the  $\alpha_{2B}$  adrenergic receptors in salt-induced hypertension, we can hypothesise that their probable action in arterial hypertension in SHR may be due to functional factors that might either act at a later stage of DNA transcription, on the cytoplasm or the cellular membrane,

or via quantitative differences, namely the density or proportion of receptor subtypes in various brain sites, or, alternatively, by disturbing the balance between  $\alpha_1$  and  $\alpha_2$  adrenergic receptors.

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