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The SHR Y chromosome increases cardiovascular, endocrine, and behavioral responses to stress compared to the WKY Y chromosome

Cherec Dickey, Jonathan Toot, Melissa Terwilliger, Rex Payne, Monte Turner, Daniel Ely *

Department of Biology, The University of Akron, Akron, OH 44325-3908, United States

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ABSTRACT

The SHR Y chromosome has loci which are involved with behavioral, endocrine and brain phenotypes and respond to acute stress to a different degree than that of the WKY Y chromosome. The objectives were to determine if WKY males with an SHR Y chromosome (SHR/y) when compared to males with a WKY Y chromosome would have: 1. a greater increase in systolic and diastolic blood pressures (BP), heart rate (HR), and locomotor activity when placed in an open field environment and during an acute stress procedure; 2. enhanced stress hormone responses; 3. greater voluntary running; and 4. increased brain Sry expression. The SHR/y strain showed a significant rise in BP (32%) and HR (10%) during the open field test and exhibited higher BP (46% change) during air jet stress. SHR/y had higher locomotor activity and less immobility and had increased stress induced plasma norepinephrine and adrenocorticotrophic hormone and 3–4× more voluntary running compared to WKY. Differential Sry expression between WKY and SHR/y in amygdala and hippocampus was altered at rest and during acute stress more than that of WKY. Evidence suggests that this animal model allows novel functions of Y chromosome loci to be revealed. In conclusion, a transcription factor on the SHR Y chromosome, Sry, may be responsible for the cardiovascular, endocrine and behavioral phenotype differences between SHR/y and WKY males.

1. Introduction

The spontaneously hypertensive rat (SHR) Y chromosome has loci that are potentially involved in many different behavioral, endocrine and brain phenotypes that are stress responsive compared to those in males with a WKY Y chromosome. We have characterized the blood pressure response and the involvement of the sympathetic nervous system but not behavioral differences between males with the different Y chromosomes. The following study presents evidence suggesting that comparing the responses of these two rat substrains with different Y chromosomes may be informative in discovering novel functions of Y chromosome loci.

The SHR is a common animal model for the study of hypertension and our laboratory has focused primarily on the contribution of the Y chromosome to hypertension [11,13,40,42]. The animal model used is a consomic rat strain that we developed through backcrossing the male offspring from an SHR father and WKY mother to a WKY mother for 19 generations [6].Genetically, this leads to a male rat with WKY autosomes and X chromosome and an SHR Y chromosome. This resulted in a borderline hypertensive rat (designated SHR/y) that has elevated indices of sympathetic nervous system (SNS) activity compared to WKY males [6]. Further research identified a Y chromosome locus, Sry which was responsible for the BP rise and is the testis determining factor. By exogenously delivering Sry via a plasmid vector and electroporation to the adrenal or kidney of normotensive WKY males, BP significantly increased, appeared to be responsible for the BP effect [5,11,12,14]. Specifically, the Sry gene is responsible for the blood pressure (BP) rise and a number of other physiological effects [14,44].

However, it has not yet been determined if the SHR Y chromosome and the Sry locus affects specific behavioral phenotypes like open field behavior and voluntary running wheel exercise that differ between SHR and WKY males [22]. Also the endocrine and brain responses to stress have not been thoroughly tested in the SHR/y animal model. Based on previous findings regarding the SHR Y chromosome and hypertension due to elevated SNS activity, we developed the following hypotheses: 1) SHR/y males compared to WKY males will exhibit elevated locomotor activity and BP to exposure in the open field; 2) increased BP and hormone response to air jet stimulation; 3) higher voluntary exercise; and 4) greater brain Sry expression to physical restraint.

2. Methods

2.1. Animals

In order to examine these multiple variables, four experiments were performed using adult male borderline spontaneously hypertensive rats (SHR/y, n = 30) and male normotensive Wistar-Kyoto (WKY,

* Corresponding author at: Dept. Biology, University of Akron, Akron, OH 44325, United States. Tel.: +1 330 9727159; fax: +1 330 9728445.
E-mail address: ely1@uakron.edu (D. Ely).

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n = 30) rats. Parental strains were originally obtained from Harlan Sprague Dawley and were determined by the National Institutes of Health (NIH) to be comparable with most genetically authentic SHR and WKY rat strains in the United States [13]. The rats were bred for more than 19 generations in order to develop substrains where the Y chromosome and the autosomal loci were separated. One of these substrains is the SHR/y rat which was used in this experiment and is genetically a WKY rat with an SHR Y chromosome [45]. Animals in all experiments were maintained housed in a home cage with food and water ad libitum and lights on a 12 h/12 h light/dark cycle (0700–1900; 1900–0700, respectively). Temperature and humidity were maintained at 27–29 °C and 50–70% respectively. All animals were treated in a humane manner according to NIH guidelines and all experiments were approved by the University of Akron IACUC. The stimulation types used in the following experiments were: open field, restraint and air jet to the face. The concept was to use several acute standardized tests that would elicit physiological changes.

2.1.1. Experiment 1: Open field behavior

The objective of this experiment was to compare the cardiovascular responses in WKY and SHR/y males in the open field and in response to air jet stimulation. Each rat (WKY: n = 4, SHR/y: n = 4, nonsiblings) was implanted with a telemetry unit (Data Sciences) two weeks before experimentation began [2]. HR, SBP, and DBP were measured via the telemetry devices every 30 min, 24 h a day. Due to the nocturnal nature of the animals, lights were automatically turned off at 1900 each night and turned back on at 0700 each morning. Experiments took place from 0800 to 1130. An open field apparatus was constructed of a Plexiglas-covered wooden base, with Plexiglas sides and a screen cover and measured approximately 1.2 m by 1.2 m with a 30 × 30 cm grid marked on the floor. Four telemetry receiver units were placed under the field, each covering a quarter of the field. Five open field trials were performed over a 3 week period on days 1, 4, 7, 15, and 21. During each trial, a number of behaviors were measured: 1) immobility behavior was recorded for any behavior in which an animal stopped moving with no movement of the head or any other extremities; 2) rearing was recorded for any time an animal picked up its front paws simultaneously in order to extend its body in the upward vertical direction, often sniffing and moving the head in a radar-like fashion; 3) grooming was used to identify an animal cleaning itself, usually using the front paws on the face and front of the body; and 4) defecation pellets were also counted. An effective acute stressor for observing and measuring emotionality-related behaviors is the open field test [24,36]. For each trial, experimentation began at approximately 0815 in the morning. Before the experiments began, each telemetry unit and receiver was operationally checked by placing a telemetered non-experimental rat into each quadrant of the open field to ensure that a strong radio signal was received. The clock in the observational room was synchronized with the computer in the next room.

Each rat's cage was moved from its position on the shelf onto a bench near the open field about 20 s before the trial for that rat was to begin. The transfer of the rat to the open field began approximately three seconds before the trial started. The animal was placed in one of four corners of the field with its tail toward the periphery and its head toward the center of the field. The trials lasted 10 min. The open field was cleaned with 50% acetic acid and allowed to dry before the trial for the next rat was to begin.

The same eight rats were used for the air jet stimulation test as were used for the open field 1 week following the last open field test. The air jet tests lasted for 30 s with BP readings taken at ten-second intervals [6]. Briefly, a stream of air from a compressed air tank was aimed at the animal's nose via a Tygon tube connected to a 5 ml disposable glass pipet. As the animal moved to avoid the air jet stream the air jet was moved to continually be aimed at the nose of the animal. The rats were kept in their cages in the open field study room. The entire cage with the rat in it was moved to the open field apparatus along with its telemetry receiver unit. After the move, the rats were allowed about 5 min before the test was to begin in order to determine a baseline. After the air jet stress, each rat was left on the receiver for another 2 min to obtain a post-trial measurement.

2.1.2. Experiment 2: Stress hormone responses

The objective of this experiment was to examine the responses of plasma norepinephrine (NE), adrenocorticotrophin hormone (ACTH) and corticosterone in each group to 1 h of restraint. Adult (15 week) male SHR/y (n = 8) and WKY (n = 8) were given 1 h of restraint and the animals did not struggle but often fell asleep. Blood samples were taken under Brevital anesthetic immediately after (50 mg/kg, IP; E. Lilly, Indianapolis, IN) with a 2–3 ml retro-orbital blood sample collected between 1100 and 1700 h and centrifuged for 5 min (5000 × *g*) to obtain plasma and stored at −70 C until analysis. Plasma NE was analyzed using high pressure liquid chromatography with electrochemical detection (Waters 460 Detector, Milford, MA, [10]).

ACTH was analyzed by RIA (DiaSorin, Stillwater, Mn). The intra-assay variation was 8.1%, the inter-assay variation was 6.7%, with a sensitivity at 15 pg/ml at the 95% confidence limit, and with the highest cross-reactivity being porcine ACTH 1–39 and human ACTH 1–24 at 100%, with other peptides at <0.01%.

Corticosterone was analyzed by RIA (DSL-80100, Diagnostic Systems Laboratories, Inc., Webster, TX.). The intra-assay variation was 3.43%, the inter-assay variation was 7.3%, with a sensitivity at 2.7 ng·ml at the 95% confidence limit, and with no reported cross-reactivity.

2.1.3. Experiment 3: Voluntary exercise

The rationale for the voluntary exercise experiment was to determine if the SHR/y increased activity in the open field would show a parallel increase with voluntary running. As part of another experiment we examined the role of the gene Sry in running behavior. Therefore, the objective of this experiment was to determine if SHR/y males would voluntarily exercise more than WKY males. Adult WKY (n = 6) and SHR/y (n = 6) males were individually placed into a standard rat cage connected with PVC pipe to a running wheel for a 9 week period. Each running wheel recorded the number of revolutions per 24 h which was converted to m/24 h [20].

2.1.4. Experiment 4: Brain Sry expression

The objective of this experiment was to determine if SHR/y males had differential brain Sry expression at rest and during restraint as compared to WKY males. Adult male WKY and SHR/y rats were used and divided into two groups (n = 5–6/group). One group had the brains analyzed at rest and the other group was restrained for 1 h for 2 consecutive days and then brains removed immediately after the second restraint. The brain regions to be studied were determined by areas that we have previously tested to be involved with blood pressure regulation, but we did not test all possible cardiovascular areas. Both the paraventricular hypothalamic nucleus and the dorsomedial hypothalamic nucleus have been implicated in cardiovascular control and studies using pharmacological blockers established that the DM but not PV hypothalamic nucleus was involved in an air jet stimulation response but we did not examine these areas [37]. The ventromedial hypothalamic (VMH) region was used because previously we examined this area in relation to brain catecholamines and the androgen receptor [12]. Also morphological differences between SHR and WKY have been established [32].Brain tissue from the following areas were dissected: basolateral amygdala, hippocampus, and ventromedial hypothalamus. All of the rats were euthanized with an overdose of pentothal and the brain removed and placed in RNA*later*® in test tubes and put into a freezer at −80 °C until specific regions were ready to be dissected. Each brain was then sectioned by hand using a razor blade. About eight sections