Vascular responses in aortic rings of a consomic rat panel derived from the Fawn Hooded Hypertensive strain

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Kunert MP, Dwinell MR, Lombard JH. Vascular responses in aortic rings of a consomic rat panel derived from the Fawn Hooded Hypertensive strain. Physiol Genomics 42A: 244–258, 2010. First published September 14, 2010; doi:10.1152/physiolgenomics.00124.2010.—The present experiments, utilizing the high-throughput vascular protocol of PhysGen (Program for Genomic Applications) characterized the responses of aortic rings to vasoconstrictor (phenylephrine) and vasodilator (acetylcholine, sodium nitroprusside, and reduced tissue bath PO2) stimuli in consomic rat strains derived from a cross between the Fawn Hooded Hypertensive rat (FHH/EurMcwi) and the Brown Norway normotensive (BN/HsdMcwi) rat. The effects of substituting individual BN chromosomes into the FHH genetic background were determined in animals that were maintained on a low-salt (0.4% NaCl) diet or switched to a high-salt (4% NaCl) diet for 3 wk. Sex-specific differences were evaluated in male and female consomic rats on similar dietary salt intake. Multiple chromosomes affected various vascular reactivity phenotypes in the FHH × BN consomic panel, and substantial salt-dependent changes in vascular reactivity and sex-specific differences in aortic reactivity were observed in individual consomic strains. However, compared with earlier studies of consomic rats derived from a cross between the BN rat and the Dahl salt-sensitive (SS) rat, only 3–7% of the vascular phenotypes were affected in a similar manner by substituting specific BN chromosomes into the FHH genetic background versus the SS genetic background. The findings of the present study stress the potential value of consomic rat panels in gaining insight into genetic factors influencing vascular reactivity and suggest that the chromosomes that appear to be involved in the determination of aortic ring reactivity in different rodent models of hypertension are highly strain- and sex specific.

Brown Norway rat; vascular reactivity; high-salt diet; chromosomal substitution

MULTIPLE STUDIES HAVE SUPPORTED the value of using chromosomal substitution (consomic strains) of experimental animals to investigate the relationship between genes and complex physiological functions (33, 34, 37, 40, 43, 59). Consomic strains are developed by the substitution of individual chromosomes from a specific strain of rat into the homogeneous genetic background of another strain, using marker-assisted selection to obtain the consomic strain of interest. This allows the contribution of individual chromosomes to the function and/or regulation of a phenotype of interest to be assessed, either by its absence from its normal genetic milieu or its presence in the genetic background of another well-characterized strain.

We previously reported (33, 34) the results of studies related to reactivity of aortic rings from a consomic panel of rat strains of both sexes developed from a cross between the Dahl salt sensitive (SS) rat (SS/JrHsdMcwi strain) and the disease-resistant Brown Norway (BN) rat (BN/HsdMcwi strain). In the present study, we report the results of high-throughput studies of aortic ring reactivity to selected vasoconstrictors and vasodilator stimuli in male and female consomic strains derived from a cross of the Fawn Hooded Hypertensive (FHH) rat (FHH/EurMcwi strain) and the BN rat (BN/HsdMcwi strain).

The FHH rat is a genetic model of spontaneous systemic hypertension, pulmonary hypertension, hypertension-induced renal damage, and a bleeding disorder caused by a platelet storage pool defect (2, 31, 32, 66). The FHH rat also has a plasma renin activity (PRA) that is 10 times higher than that in the BN rat (40). Of the relatively few studies of vascular function in the FHH rat, the overwhelming majority have investigated the control of renal (49, 60, 64, 65) and pulmonary (2–4, 46) vessels because of the sensitivity of the FHH rat to renal disease and pulmonary hypertension. However, because the FHH rat is also a model of spontaneous systemic hypertension (31, 32), it is important to gain an increased understanding of genetic factors controlling systemic vascular reactivity in this rat strain.

Isolated aortic ring preparations have been used extensively to evaluate vascular function in health and disease, and are especially well suited for high-throughput studies of vascular reactivity. Recent studies utilizing a panel of consomic rats derived from a cross between the SS rat and the BN rat revealed that the reactivity of aortic rings to different vasoactive stimuli can be affected by genes on many different chromosomes (33, 34) and that the specific chromosomes affecting aortic reactivity to specific vasoactive stimuli not only can vary with dietary salt intake (33, 34) but also can be different in male and female consomic rats (34). The present study is the first of its kind to extensively describe aortic ring reactivity of male and female FHH rats fed either a low-salt (0.4% NaCl) or a high-salt (4.0% NaCl) diet and to employ consomic strains derived from a cross between FHH and BN rats to provide initial insight into the contribution of individual FHH chromosomes to the response of aortic rings to widely studied vasoactive stimuli including the α-adrenergic agonist phenylephrine (PE), the endothelium-dependent vasodilator acetylcholine (ACh), the nitric oxide (NO) donor sodium nitroprusside (SNP), and the physiological vasodilator stimulus of reduced oxygen availability.

METHODS

Experimental Animals

All rats (low-salt female n = 224, low-salt male n = 287, high-salt female n = 281, high-salt male n = 310) were bred and housed at the Medical College of Wisconsin Animal Resource Transgenic Barrier Facility. Rats were cared for according to established National Institutes of Health guidelines for the care and use of laboratory animals.
The rats were part of a consomic rat panel in which each full-length chromosome (>95% of the chromosome) from the inbred BN/NHsdMcwi rat was substituted one at a time into the homogeneous background of the FHH/EurMcwi rat. The consomic strains are formally designated as FHH-N\textsuperscript{BN} [FHH-\textsuperscript{BN} throughout the text, where \textit{n} designates the substituted chromosome (i.e., FHH-\textsuperscript{1BN}, FHH-\textsuperscript{2BN}, etc)]. Complete genotypes of each consomic rat are listed on the PhysGen [Program for Genomic Applications (PGA)] website (http://pga.mcw.edu).

Rats used in the present PhysGen study were housed in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited animal care facility, and all procedures were approved by the Medical College of Wisconsin Institutional Animal Care and Use Committee. All rats were maintained from birth on 0.4% NaCl Teklad chow (3075S; Madison, WI). Three weeks before the study, at 7 wk of age, one group of consomic rats (10 female and 10 male rats of each consomic strain and 2 male parental FHH rats for sentinels) were placed on a high-salt (4.0% NaCl) diet (Teklad, TDD1454). Another group of consomic rats (10 females, 10 males) and two male FHH sentinels of the same strain and number were maintained on the low-salt (0.4% NaCl) Teklad diet for the entire experimental period. Aortic rings from two male FHH sentinel rats were studied simultaneously with the aortic rings of each consomic strain to confirm the internal consistency of the experimental protocol from strain to strain and week to week, since 10 male rats of the same strain and stressor were studied at the same time. Phenotypes were measured in four groups of FHH parental rats at four time periods over the year to account for any seasonal variations.

Experimental Protocol

Equipment used in these studies included a 16-tissue bath system with reservoirs and circulators (Radnoti Glass Technology, Monrovia, CA), model 410 tissue force analyzers (TFAs) (Micro-Med, Louisville, KY), and Grass FT-03 force transducers (Grass Instruments, Rockland, MA). Gas tanks for delivery of 95%, 21%, 10%, 5%, and 0% oxygen mixtures (with 5% CO\textsubscript{2}-balance N\textsubscript{2}) were obtained from Praxair (Burlington, WI). The 10-wk-old rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg) to produce a deep anesthesia. The chest of the rat was opened, and a 3- to 5-cm length of aorta was removed and placed in a labeled petri dish containing room temperature physiological salt solution (PSS) (in mM: 119 NaCl, 4.7 KCl, 1.17 MgSO\textsubscript{4}, 7.0 H\textsubscript{2}O, 1.6 CaCl\textsubscript{2}, 2.0 H\textsubscript{2}O, 1.18 Na\textsubscript{2}HPO\textsubscript{4}, 24 NaHCO\textsubscript{3}, 0.03 EDTA, 5.5 glucose, and 5.0 HEPES). Two aortic rings were used from each rat. With few exceptions, rings studied in the relaxation protocols were from the same animals studied for the contraction experiments. Ten female and ten male rats from each strain were studied within 1 wk (maximum of 8 rings per day). The rings were attached to a Grass FT03 force transducer connected to a TFA and mounted in fresh PSS for each rat. One ring was mounted in the eight-bath setup for the contraction and hypoxia studies, and another was mounted in the eight-bath setup for the relaxation studies.

General Procedures for Aortic Ring Studies

The tension on the rings was adjusted to 1.5 g of passive force and allowed to equilibrate for 30 min in the bath with a 21% O\textsubscript{2}, 5% CO\textsubscript{2}, 74% N\textsubscript{2} gas mixture. To process the aortic ring studies in the high-throughput fashion required for the present studies, the initial passive tension on the rings was set at 1.5 g, which is in the middle of the range of reported values in the literature (0.4–3.0 g) for similar studies of aortic ring preparations. However, it is important to stress the need for precise normalization of passive force in any follow-up studies of vascular reactivity in aortic rings from any of the strains used in the present study, because differences in arterial blood pressure in the individual strains and possible differences in wall structure and mechanics could affect the length-tension relationship in the aorta and therefore the optimum passive stretch on the aorta in those individual strains.

The rings were washed with fresh PSS every 10 min. Passive force was readjusted to 1.5 g as needed during this period. When passive force on the rings was stable, the baseline reading on the tissue force analyzers was set at 0 g. PE, at a final concentration of 10\textsuperscript{-7} M, was added to the bath to contract the ring, and force was allowed to stabilize for 5 min. ACh, at a final concentration of 10\textsuperscript{-5} M, was then added to the precontracted rings for 5 min to test for endothelial integrity. If a ring failed to contract in response to PE or failed to relax in response to ACh, it was replaced with a different aortic ring from the same rat.

After the initial test for vessel viability and endothelial integrity, the ring was washed three times with PSS, allowed to equilibrate, and then rewashed with fresh PSS at 10-min intervals until the measured active force stabilized at 0 g. The maximum contraction achievable by the ring was then determined by filling the bath with 80 mM K\textsuperscript{+} and adding PE at a final concentration of 10\textsuperscript{-6} M. Maximal contractile force generated in response to the combination of 80 mM K\textsuperscript{+} and 10\textsuperscript{-5} M PE was normalized to the wet weight of the aortic ring (determined at the end of the experiment). After the maximum contraction of the aortic rings was determined, the vessels were allowed to stabilize and washed with PSS every 10 min until the measured active force returned to 0 g.

Contraction Protocol

Cumulative concentration-response curves to PE, an \(\alpha\)-adrenergic agonist that is widely used to test adrenergic vasoconstrictor sensitivity in isolated blood vessels, were created by increasing the PE concentration in the tissue bath by successive addition of appropriate dilutions of stock solutions to achieve final bath concentrations of 10\textsuperscript{-7} M to 3 \times 10\textsuperscript{-4} M PE. The rings were then washed with PSS, allowed to equilibrate, and rewashed with fresh PSS at 5- to 10-min intervals until active force returned to a stable value of 0 g.

ACh and SNP Protocol

In the relaxation protocol, aortic rings were precontracted with 10\textsuperscript{-7} M PE, and cumulative concentration-response curves to ACh were created by increasing the ACh concentration in the tissue bath by successive addition of appropriate dilutions of stock solutions to achieve final bath concentrations of 10\textsuperscript{-9} to 3 \times 10\textsuperscript{-5} M ACh. Cumulative concentration-response curves to SNP were created by increasing the SNP concentration in the tissue bath by successive addition of appropriate dilutions of stock solutions to achieve final bath concentrations of 10\textsuperscript{-10} to 3 \times 10\textsuperscript{-6} M SNP [for typical concentration-response curves for ACh and SNP see Kunert et al. (33)].

Hypoxic Relaxation Protocol

A variety of studies have demonstrated that vascular sensitivity to changes in oxygen availability is significantly altered in many forms of hypertension (13, 14, 52) and by elevated dietary salt intake, independent of an elevation in arterial blood pressure (38, 69). With this in mind, we tested the sensitivity of aortic rings to stepwise reductions in tissue bath PO\textsubscript{2} in a subset of the consomic strains. The aortic rings used for this hypoxic relaxation protocol were the same ones used for the PE contraction protocol in those strains. In those studies, the rings were precontracted with PE at a final concentration of 10\textsuperscript{-7} M and allowed to stabilize at a maximum response (\(\approx\)10 min). Then the gas equilibration mixture in the tissue bath was changed from 21% O\textsubscript{2}, 5% CO\textsubscript{2}, 74% N\textsubscript{2} to a mixture containing 95% O\textsubscript{2},5 %C O\textsubscript{2}. The switch to 95% O\textsubscript{2} was initially intended to determine whether aortic rings generate more contractile force when PO\textsubscript{2} is elevated but was subsequently discarded as a phenotype of interest. After 10 min at 95% O\textsubscript{2}-5% CO\textsubscript{2}, the oxygen concentration in the bath was reduced.
in a stepwise fashion at 20-min intervals to mixtures containing 10% O2, 5% O3, and 0% O4, with 5% CO2 and the balance N2. The volume of each tissue bath was small (5 ml); therefore, equilibration with the gases required only a few minutes. To verify that the ring was viable at the end of the hypoxic relaxation protocol, the bath was returned to 95% O2-5% CO2 for 20 min. If the aortic ring did not contract and develop a force approximately equal to the initial force development in response to 95% O2, the force values obtained during exposure to hypoxia were eliminated from the analysis. Vessel responses to reduced PO2 and SNP (see above) were only measured in a subset of the consomic strains in order to expedite the study of large groups of rats demanded by the high-throughput design of the studies.

Data and Statistical Analyses

Real-time analog-to-digital conversions of force waveforms and digital processing for waveform characteristics were accomplished with a Digi-Med System model 410 TFA (Micro-Med). The resultant data were automatically transferred into a spreadsheet for statistical analyses with GraphPad Prism software. Sensitivity to each agent was expressed as the negative log of EC50, based on maximum contraction (PE) or percentage of maximum possible relaxation from the force generated during initial preconstriction of the aorta with PE for the vasodilator stimuli. EC50 values were directly calculated via curve fitting in the GraphPad Prism program.

A robust iterative digital algorithm in the TFA automatically determined the lowest inflection point for the time-dependent slope in the PE-induced precontraction curve. The “straight-line slope” (in g/min) from that lowest inflection point to the start of the precontraction curve was used as the best quantitative indicator of the speed of the mechanisms that drive the initial, rapidly developing component of the PE-induced contraction. These “fast mechanisms” include Ca2+ release from intracellular calcium stores (sarcoplasmic reticulum), myosin ATPase activity, and the rate of cross-bridge cycling, which is related to the degree of phosphorylation of the regulatory light chain on the myosin molecule.

The TFA iterative algorithm also automatically determined the highest inflection point for the later portion of the time-dependent slope in the PE-induced precontraction curve. The straight-line slope (in g/min) from that highest inflection point to the start of the precontraction curve (termed the “slow slope”) was used as the best quantitative indicator of the effect of the mechanisms that drive the slowly developing component of contraction after the initial rapidly developing component of PE-induced contraction. These “slow mechanisms” include Ca2+ influx from the extracellular fluid, the balance between myosin light chain kinase and myosin light chain phosphatase activity, and any other factors that affect myofilament Ca2+ sensitivity.

All data are summarized as means ± SE. Differences between means were assessed by conventional ANOVA or, if Levene’s test showed that the groups had unequal variances, by a nonparametric ANOVA. This was followed by Dunnett’s test to compare all consomic and congenic strains to PE (expressed as the negative log of the EC50), based on maximum contraction (PE) or percentage of maximum possible relaxation from the force generated during initial preconstriction of the aorta with PE for the vasodilator stimuli. EC50 values were directly calculated via curve fitting in the GraphPad Prism program.

RESULTS

For the sake of clarity in Figs. 1–7, we present only the data that were significantly different from those of rats of the same sex in the FHH parental strain. The data from the BN rats that we report in this study are the same data that we reported in two previous articles (33, 34) comparing the BN rat with the parental Dahl SS rat and the SS × BN consomic strains. Consistency of phenotypes over time in vascular reactivity studies in the PhysGen protocol was verified by including two sentinel rats in each vascular phenotyping experiment throughout the duration of the project.

Differences in Aortic Ring Sensitivity to Vasoactive Stimuli in BN and Consomic Strains vs. Parental FHH Strain of Same Sex

Phenylephrine. The sensitivity of the aortic rings from the male and female FHH and BN parental strains and the derived consomic strains to PE (expressed as the negative log of the EC50) are summarized in Fig. 1. When male rats were maintained on a low-salt diet, aortic rings of male FHH-10BN rats were more sensitive to PE (higher negative log of EC50) and those of the BN parental strain were less sensitive to PE (lower negative log of EC50) than those of the male FHH parental rats (Fig. 1A). Aortic rings of the male BN parental rats and female consomic strain FHH-11BN, -14BN, -5BN, -2BN, -1BN, -8BN, and -15BN maintained on a low-salt diet were less sensitive to PE than those of the female parental FHH rats (Fig. 1A).

When the male rats were maintained on a high-salt diet, aortic rings of the FHH-12BN and -19BN rats were more sensitive to PE (higher negative log EC50) than those of the parental FHH rats (Fig. 1B), while aortic rings of the male BN rats were less sensitive to PE (lower negative log EC50) than those of males of the parental FHH strain. The aortic rings of the female FHH-9BN rats fed the high-salt diet were more sensitive to PE than those of the female FHH parental strain (Fig. 1B).

Fast and slow slopes of PE-induced contraction. The fast and slow slopes of PE-induced contractions (g/min) are summarized in Figs. 2 and 3, respectively. When the rats were fed a low-salt diet (Fig. 2A), the fast slope of the PE curve was significantly higher in both male and female BN parental rats than in male or female FHH parental rats, respectively. The fast slope of the PE curve was significantly lower in the male FHH-5BN rats than in the male FHH parental rats. When the rats were fed a high-salt diet (Fig. 2B), the fast slope of the PE curve was higher in the male BN parental strain than in the male FHH parental strain. High-salt diet had no effect on fast slope of PE-induced contraction in females.

When the rats were fed a low-salt diet, the slow slope of the PE curve (Fig. 3) was significantly higher in the male BN parental rats and FHH-7BN rats than in the male FHH parental strain and significantly lower in the male FHH-5BN rats than in male FHH parental rats. In the female rats, the slow slope of PE-induced contraction was significantly higher in the FHH-7BN rats than in the female FHH parental strain. There were no significant differences between the slow slopes of the PE response in any of the experimental groups when the animals were fed a high-salt diet.

Maximum force. The maximum force (normalized to wet weight) attained by the aortic rings of the male and female FHH and BN parental strains and the derived consomic strains during contraction to 80 mM K+ + 10−5 M PE are summarized in Fig. 4. Under low-salt conditions (Fig. 4A) the aortas of the male and female parental BN rats achieved a greater maximum force per unit wet weight than those of FHH parental...
rats of the same sex. The aortic rings of the male FHH-3BN, -2BN, and -YBN rats maintained on a low-salt diet produced a greater force per unit wet weight than those of the male FHH parental strain, while those of the FHH-5BN and -15BN rats attained a lesser force than the male parental FHH strain. Aortas from female FHH-15BN rats fed a low-salt diet produced a significantly lower maximum force than those of the female FHH parental strain. When the rats were maintained on a high-salt diet (Fig. 4B), the aortic rings of the male BN parental rats, as well as male FHH-19BN and FHH-1BN rats, attained a greater maximum force than those of the male FHH parental rats. Aortic rings from the female FHH-14BN, -XBN, -15BN, and -11BN rats fed a high-salt diet produced a significantly lower maximum force per unit wet weight than those of the female FHH parental rats.

### Acetylcholine

The sensitivity of the aortic rings from the male FHH and BN parental strains and the derived consomic strains to ACh (expressed as negative log of EC50) are summarized in Fig. 5. When the rats were fed a low-salt diet, aortic rings from males of the FHH-14BN, -5BN, -4BN, -11BN, -12BN, -3BN, and -YBN consomic strains were more sensitive (higher negative log EC50) to ACh than those of the male FHH parental strain, while aortic rings of male consomic FHH-10BN rats were less sensitive to ACh (lower negative log EC50).

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**Fig. 1.** Comparison of the sensitivity to phenylephrine (PE, $-\log{EC_{50}}$) of aortic rings from male and female consomic rats to the Fawn Hooded Hypertensive (FHH) parental rat of the same sex after the rats were fed a low-salt (LS) diet (0.4% NaCl; A) or a high-salt (HS) diet (4.0% NaCl; B) for 3 wk. Data are summarized as means ± SE. *P < 0.05, significantly different from FHH parental strain. In this and subsequent figures, numbers, X, and Y under columns indicate Brown Norway (BN) chromosome introgressed into FHH background in consomic strain.

**Fig. 2.** Comparison of the fast slope of the PE contraction of aortic rings from male and female consomic rats to the FHH parental rat of the same sex after the rats were fed a low-salt diet (0.4% NaCl; A) or a HS diet (4.0% NaCl; B) for 3 wk. Data are summarized as means ± SE. *P < 0.05, significantly different from FHH parental strain.
the rats were fed a high-salt diet, aortic rings from male parental BN rats and male consomic FHH-3BN rats were less sensitive to ACh than those from the male FHH parental strain, while aortic rings from FHH-5BN rats were significantly more sensitive to ACh than those from parental male FHH rats. There were no differences in ACh sensitivity among the female rats fed either diet.

**Maximum relaxation to ACh.** The maximum relaxations in response to ACh that were attained by aortic rings from male and female FHH and BN parental strains and the derived consomic strains are summarized in Fig. 6. Under low-salt conditions (Fig. 6A) aortas from male consomic FHH-5BN rats relaxed by a greater percentage than those of the male FHH parental strain. Aortic rings of female FHH-5BN, -15BN, and -17BN consomic rats relaxed significantly more to ACh than those of female parental FHH rats. When rats were fed a high-salt diet (Fig. 6B), aortic rings from male consomic FHH-1BN rats relaxed significantly less to ACh than those of male FHH parental rats, while aortic rings of male FHH-6BN rats relaxed significantly more to ACh than those of the FHH parental rats. Aortic rings of female FHH-XBN rats exhibited a greater maximum relaxation to ACh than those of the female FHH parental strain.

**SNP.** Aortas from male and female rats of all strains that were tested relaxed in response to the NO donor SNP. There were no significant differences in vessel responses to SNP among males or females with either low-salt or high-salt diet.

**Reduced Po2.** Figure 7 summarizes the extent of relaxation of the aortic rings of male and female parental and derived consomic strains to three different levels of reduced Po2 in the tissue bath (10% O2, 5% O2, and 0% O2). When rats were fed a low-salt diet, there were no differences in aortic relaxation to 10% O2, 5% O2, or 0% O2 in male or female consomic strains compared with aortic rings of parental FHH rats of the same sex. When rats were fed a high-salt diet, aortic rings of male and female BN parental rats relaxed significantly more to 10% O2 than those of parental FHH rats of the same sex, and aortic rings of male BN rats also relaxed significantly more to 5% O2 than those of the FHH parental strain (Fig. 7). Aortic rings of male consomic FHH-1BN rats fed a high-salt diet relaxed significantly less to 10% O2 than aortic rings from FHH parental rats.

**Effect of High-Salt Diet on Aortic Ring Sensitivity Within Strains**

Table 1 summarizes the effects of high-salt diet on contractile parameters and the sensitivity of aortic rings of male rats to
different vasoactive stimuli within strains. In these experiments, high-salt diet decreased aortic sensitivity to PE in male FHH-1BN and FHH-10BN rats and increased sensitivity to PE in male FHH-5BN, -11BN, -12BN, -13BN, and -19BN consomic rats. High-salt diet also increased the fast and slow slopes of PE-induced contraction in male FHH-5BN rats and decreased the slow slope in male FHH-6BN rats. Maximum force of contraction was decreased by high-salt diet in male FHH-2BN, -17BN, and -YBN rats in parental BN rats and increased in male FHH-5BN and -16BN rats.

Aortic ring sensitivity to ACh was reduced in males of the FHH-3BN, -11BN, and -17BN strains and increased in male FHH-7BN and -10BN consomic rats when the rats were fed a high-salt diet. Under high-salt conditions, maximum relaxation to ACh was increased in FHH-1BN, -3BN, -5BN, and -17BN rats, while maximum relaxation to ACh was increased in FHH-6BN rats. Sensitivity to 10% O2 and 0% O2 in the tissue bath was lower in parental FHH rats and FHH-1BN consomic rats, and sensitivity to equilibration of the tissue bath with 0% O2 was decreased in male FHH-12BN rats fed a high-salt diet compared with animals fed a low-salt diet. Sensitivity to 5% O2 was increased in male BN parental rats fed high-salt diet compared with BN rats maintained on low-salt diet. High-salt diet had no effect on SNP sensitivity in any of the strains tested.

Table 2 summarizes the effects of a high-salt diet on contractile parameters and the sensitivity of the aortic rings of female rats to different vasoactive stimuli within strains. Aortic sensitivity to PE was decreased in female FHH-10BN, -18BN, and -XBN consomic rats when the animals were fed a high-salt diet. High-salt diet increased aortic sensitivity to PE in male FHH-11BN consomic rats. The fast slope of PE-induced contraction was increased by high-salt diet in female FHH-15BN and reduced in female FHH-16BN and -18BN rats. The slow slope of PE-induced contraction was increased by high-salt diet in FHH-5BN, -13BN, and -15BN rats and decreased in FHH-18BN and female parental BN rats. High-salt diet increased the maximum force per unit weight attained by aortic rings from female FHH-1BN and -5BN rats and reduced maximum contractile force in aortic rings from female FHH-11BN and -18BN rats. Aortic sensitivity to ACh was decreased by high-salt diet in female FHH-1BN and -5BN rats and reduced maximum contractile force in aortic rings from female FHH-11BN and -18BN rats. Aortic sensitivity to ACh was increased in high-salt diet in female FHH-11BN and -14BN rats and increased in aortic rings from female FHH-16BN and -19BN rats. High-salt diet also decreased the maximum relaxation to ACh in female FHH-1BN rats and increased maximum relaxation to ACh in FHH-16BN and -XBN rats. There were no salt-induced changes in aortic sensitivity to SNP or to the three levels of reduced PO2 in the female rats.

**Fig. 5.** Comparison of the sensitivity to acetylcholine (ACh, logEC50) of aortic rings from male and female consomic rats compared with those of the FHH parental strain after the rats were fed a LS diet (0.4% NaCl; A) or a HS diet (4.0% NaCl; B) for 3 wk. Each ring was precontracted with PE (0.1 μM). Data are summarized as means ± SE. *P < 0.05, significantly different from FHH parental strain.

**Fig. 6.** Comparison of the maximum relaxation (%) to ACh in PE (0.1 μM)-contracted aortic rings from male and female consomic rats compared with those from the FHH parental strain of the same sex after the rats were fed a LS diet (0.4% NaCl; A) or a HS diet (4.0% NaCl; B) for 3 wk. Data are summarized as means ± SE. *P < 0.05, significantly different from FHH parental strain.
Sex-Specific Differences in Aortic Ring Sensitivity Within Strains (Low-Salt Diet)

Table 3 summarizes the differences in contractile parameters and aortic ring sensitivity to PE, ACh, SNP, and three levels of reduced bath PO2 (10% O2, 5% O2, and 0% O2) in females fed a low-salt diet compared with rings from males of the same strain fed a low-salt diet. These comparisons of phenotypes revealed several sex-specific differences in both contractile and vasodilator responses within strains. Aortic rings of female FHH-4BN, -12BN, and -18BN rats were more sensitive to PE than those of their male counterparts. The fast slope of the PE curve was reduced in the female consomic FHH-12BN and -17BN rats and the parental BN rats, compared with males of the corresponding strains. The slow slope of the PE curve was lower in female FHH parental rats and the FHH-4BN, -5BN, -6BN, -11BN, -12BN, -16BN, -18BN, and -XBN consomic strains was significantly greater than that attained by aortic rings from male rats of the corresponding strains. The maximum force attained by aortic rings of female FHH parental rats and the FHH-4BN, -5BN, -6BN, -11BN, -12BN, -16BN, -18BN, and -XBN consomic strains was significantly greater than that attained by aortic rings from male rats of the corresponding strains. Sensitivity to ACh was lower in aortic rings from female consomic FHH-10BN rats than in the males fed a high-salt diet. Maximum relaxation to ACh was greater in female FHH-17BN consomic rats and less in female FHH-20BN consomic rats than in males of the same strain. Finally, aortic sensitivity to SNP was greater in the female parental BN rats compared with the male BN rats fed a high-salt diet. There were no sex-specific differences in aortic sensitivity to reduced PO2 in any of the strains fed a high-salt diet.

Sex-Specific Differences in Aortic Ring Sensitivity Within Strains (High-Salt Diet)

Table 4 summarizes the within-strain differences in the same parameters in female rats fed a high-salt diet (4.0% NaCl) compared with males of the same strain that were fed a high-salt diet. Aortic rings of female parental FHH rats were less sensitive to PE than those of the males, while aortic rings of female consomic FHH-1BN rats fed a high-salt diet were more sensitive to PE than those of male FHH-1BN rats. The fast slope of the PE curve was significantly less in the female consomic FHH-1BN rats than in the males fed a high-salt diet. The maximum force reached by aortic rings of female FHH-1BN, -2BN, -5BN, -10BN, -17BN, and -20BN consomic rats and parental BN rats was significantly larger than that generated by aortic rings from males of the same strain. Sensitivity to ACh was significantly higher in female FHH-3BN rats fed a high-salt diet than in male FHH-3BN rats. Maximum relaxation to ACh was greater in female FHH-17BN consomic rats and less in female FHH-20BN consomic rats than in males of the same strain. Finally, aortic sensitivity to SNP was greater in the female parental BN rats compared with the male BN rats fed a high-salt diet. There were no sex-specific differences in aortic sensitivity to reduced PO2 in any of the strains fed a high-salt diet.

DISCUSSION

Extensive studies in the literature have reported quantitative trait loci (QTLs) for a variety of physiological and pathophysiological phenotypes in different rat models of hypertension (12, 17, 18, 26, 55, 57, 58, 61, 63, 72–75, 77). A substantial number of other studies have utilized consomic rat models to evaluate the contribution of specific chromosomes to physiological regulatory mechanisms and to the development of pathological conditions including hypertension (7–9, 15, 16, 22, 24, 29, 30, 33, 34, 37, 39–42, 47, 48, 70). Relevant to consomic approaches in FHH rats, a recent study by Mattson and coworkers (40) used the FHH × BN consomic panel to investigate the effect of substituting each of the BN chromosomes individually into the FHH background in rats fed a high-salt (8% NaCl) diet with N(G)-nitro-L-arginine methyl ester (L-NAME) in the drinking water to produce hypertension and renal disease. In those studies, the authors found evidence that genes on chromosomes 1, 15, 16, 18, and 20 can modify salt + L-NAME-induced hypertension and proteinuria. Although conscious blood pressure and renal function were not measured in...
These individual rats, blood pressure and renal function were measured in these strains with a separate protocol and have been published previously (40). Additional blood pressure and renal function data are available on the PhysGen website (http://pga.mcw.edu).

The present studies add to existing knowledge of vascular regulation and sex-based differences in consomic rats from the FHH/H11003 BN cross by providing a comparison of phenotypes directly related to aortic ring reactivity in male and female rats maintained on a low-salt diet or exposed to a smaller increase

Table 1. Changes in sensitivity of aortic rings of male rats fed high-salt or low-salt diet

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<th>PE Sens</th>
<th>Fast Slope PE</th>
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<th>Maximum Force</th>
<th>ACh Sens</th>
<th>Maximum Relaxation to ACh</th>
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Data are changes in sensitivity of aortic rings of male rats fed a high-salt (HS, 4.0% NaCl) diet compared with those of male rats of the same strain maintained on a low-salt (LS, 0.4% NaCl) diet. Sensitivity within strain and male sex, HS diet vs. LS diet: ▼ = HS less sensitive than LS; ▲ = HS more sensitive than LS. 1-20, X, and Y indicate consomic strains in which Brown Norway (BN) rat chromosome 1-20, X, or Y was substituted into homogeneous background of Fawn Hooded Hypertensive (FHH) rat. Sens, sensitivity; PE, phenylephrine; ACh, acetylcholine; SNP, sodium nitroprusside.

Table 2. Changes in sensitivity of aortic rings of female rats fed high-salt or low-salt diet

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<th>PE Sens</th>
<th>Fast Slope PE</th>
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<th>Maximum Force</th>
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<th>Maximum Relaxation to ACh</th>
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Data are changes in sensitivity of aortic rings of female rats fed a HS (4.0% NaCl) diet compared with those of female rats of the same strain maintained on a LS (0.4% NaCl) diet. Sensitivity within strain and female sex, HS diet vs. LS diet: ▼ = HS less sensitive than LS; ▲ = HS more sensitive than LS.
in dietary salt intake (4% NaCl) in the absence of chronic L-NAME treatment to promote hypertension and renal disease. The results of this study provide initial insight into role of individual chromosomes of the FHH and/or BN rat in regulating the response of aortic rings to widely studied vasoactive stimuli including the $\alpha$-adrenergic agonist PE, the endothelium-dependent vasodilator ACh, the NO donor SNP, and the physiological vasodilator stimulus of reduced $PO_2$. In the present study, substitution of various BN chromosomes onto the homogeneous genetic background of the FHH rat modified the response of aortic rings to several vasodilators and to PE (compared with the parental FHH strain) and led to changes in aortic ring reactivity within strains when the rats were maintained on a high-salt diet for 3 wk.

Table 3. Sensitivity of aortic rings of male versus female rats of same strain maintained on low-salt diet

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<th></th>
<th>Fast PE Sens</th>
<th>Slow Slope PE</th>
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<th>ACh Sens</th>
<th>Maximum Relaxation to ACh Sens</th>
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Data indicate sensitivity of aortic rings of male vs. female rats of the same strain maintained on a LS (0.4% NaCl) diet. Comparison of low-salt female vs. low-salt male: $\downarrow$ = males less sensitive than females, $\uparrow$ = males more sensitive than females.

Table 4. Sensitivity of aortic rings from male versus female rats of same strain fed high-salt diet for 3 wk

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<th>Fast PE Sens</th>
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Data indicate sensitivity of aortic rings of male vs. female rats of the same strain fed a HS (4.0% NaCl) diet for 3 wk. Comparison of high-salt female vs. high-salt male: $\downarrow$ = males less sensitive than females, $\uparrow$ = males more sensitive than females.
The present findings corroborate the overall results of our previous work (33, 34) utilizing consomic rat strains in which individual chromosomes from the BN rat were introgressed into the SS genetic background, insofar as substitution of a number of BN chromosomes into the FHH background affected vascular reactivity in the resulting consomic rats. The results of this study are also consistent with reports indicating that different chromosomes often appear to be involved in the regulation of vessel responses to vasoactive substances (and other physiological responses) in males and females (33, 34, 44) and with existing studies of aortic vascular reactivity in SS-derived consomic rat strains (34) showing that many of the effects of a high-salt diet on aortic ring reactivity are also sex specific.

**Comparison of Specific Chromosomal Substitutions vs. Known QTLs**

In light of the studies of consomic and congenic rat strains cited above, several chromosomes are of particular interest for QTLs related to blood pressure, namely chromosomes 1, 5, and 10. In the present study, chromosomes 1 and 5 had multiple effects related to aortic sensitivity to vasoactive agonists and reduced Po2. These included effects on PE sensitivity, ACh response, and salt-dependent changes in Po2 responses (Table 1). On the other hand, chromosome 10 had relatively few effects on aortic ring reactivity in the specific consomic strains employed in this study.

Introgression of BN chromosome 1 into the FHH background is of significant interest in the FHH × BN panel because substitution of this chromosome into the FHH background not only reduces urinary protein and albumin excretion in FHH-1<sup>BN</sup> consomic rats and ameliorates salt-induced glomerular damage in FHH parental rats (40, 42) but also ameliorates hypertension in FHH-1<sup>BN</sup> consomic rats compared with FHH parental rats (40) and restores the ability of FHH to autoregulate renal blood flow that is impaired in FHH rats (39, 68) Multiple blood pressure QTLs have been reported on chromosome 1 in studies of Dahl SS rats and congenic strains derived from crossing Dahl and Lewis rats (26, 55), in Milan hypertensive rats (75), and in congenic and reciprocal congenic strains for spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats (17, 18). There is also a QTL for salt sensitivity on chromosome 1 of female Sabra hypertensive rats (72, 73).

In the present study, introgression of BN chromosome 1 into the FHH background had multiple effects on aortic reactivity in the FHH-1<sup>BN</sup> consomic rats. These included the following: 1) salt-dependent effects on ACh-induced relaxation, PE sensitivity, and sensitivity to reduced Po2 in male rats (Table 1); 2) salt-dependent effects on ACh-induced relaxation and maximum force generation in female rats (Table 2); and 3) sex-specific effects on ACh-induced relaxation and maximum contractile force generation in FHH-1<sup>BN</sup> rats fed a high-salt diet (Table 4). By contrast, introgression of BN chromosome 1 into the SS genetic background affected only two phenotypes in the SS × BN consomic panel (34), namely, sex-specific increases in ACh sensitivity and maximum force in male versus female SS-1<sup>BN</sup> rats fed a low-salt diet, with no salt-dependent or sex-specific effects in male and female rats fed a high-salt diet. Taken together, these observations indicate that there are substantial differences in the mechanisms regulating arterial reactivity to vasoactive agents in these two rodent models of hypertension, and that chromosome 1 may contain genes that play a major role in regulating arterial sensitivity to vasoactive agonists in the FHH model of spontaneous hypertension.

Chromosome 5 has been shown to have a QTL for arterial pressure in F<sub>2</sub> intercrosses of both SHR and WKY rats (74, 77) and Dahl SS and Lewis rats (12). Two closely linked interactive QTLs for arterial pressure have been reported on chromosome 5 in congenic rats in which Lewis alleles are introgressed into the SS genome (19, 36), and Roman and coworkers (54) have reported that transfer of regions of chromosome 5 containing cytochrome P-450–4A ω-hydroxylase (CYP4A) genes from the Lewis rat into the SS rat improves pressure diuresis and opposes the development of hypertension in the Dahl SS rat. There is also a highly significant QTL on chromosome 5 for infarct volumes in stroke-prone SHR rats in a SHRsp × WKY cross (25).

In the present study, introgression of chromosome 5 into the FHH genetic background led to increased maximum contractile force in all four comparisons (Tables 1–4); salt induced increases in PE sensitivity and in the fast and slow slopes of PE-induced contraction in male FHH-5<sup>BN</sup> consomic rats (Table 1) and a reduced maximum relaxation to ACh in male FHH-5<sup>BN</sup> consomic rats (Table 1), with little effect in females. These effects on vessel reactivity following introgression of BN chromosome 5 into the FHH genetic background suggest that a gene or genes on chromosome 5 may play a role in regulating vascular reactivity in the FHH model of hypertension, where there is much less information regarding the nature, mechanisms, and implications of altered reactivity in arteries outside the renal and pulmonary vascular beds. While the identity of the genes affecting vascular reactivity on chromosome 5 remains to be determined, potential candidates are genes for CYP4A, because alleles for the CYP4A2 isoform cosegregate with blood pressure in an SS × Lewis cross (62). Vascular CYP4A enzymes are also upregulated with elevated dietary salt intake (67, 68), and their vasoconstrictor metabolite 20-hydroxyeicosatetraenoic acid (20-HETE) has been implicated in enhanced reactivity of mesenteric resistance arteries (76) and skeletal muscle microvessels (35, 68) in SHR and Dahl SS rats fed a high-salt diet.

Chromosome 10 is also of potential interest in the FHH × BN consomic panel, because introgression of BN chromosome 10 into the FHH genetic background significantly increases the severity of renal disease in female FHH-10<sup>BN</sup> rats (40). Suggestive QTLs for arterial pressure and renal blood flow responses to ACh have been reported on chromosome 10 of male rats derived from an F<sub>2</sub> intercross between the SS and BN parental strains (63). In addition, several authors (20, 56, 58, 61) have reported QTLs affecting blood pressure on chromosome 10 of congenic rat strains carrying alleles from the Lewis rat and the Milan normotensive rat in the Dahl SS genetic background, and Zagato and coworkers (75) reported a QTL for systolic blood pressure on chromosome 10 in a cross between Milan hypertensive and normotensive strains. Other investigators have reported multiple interacting QTLs for blood pressure on chromosome 10 in Dahl SS rats (6) and have provided evidence that the multiple QTLs for blood pressure on rat chromosome 10 correspond to similar blood pressure QTLs on mouse chromosome 11 and human chromosome 17.
(78), supporting the value of rat genomic studies in increasing our understanding of human hypertension. However, in contrast to the multiple studies reporting QTLs for blood pressure on chromosome 10 of other strains, the effects of substituting BN chromosome 10 into the FHH background were less extensive than the effects of substituting BN chromosome 1 or 5 into the FHH genetic background (Table 1–4). The latter finding suggests that genes on chromosome 10 are less important in regulating vascular reactivity in FHH rats than genes on chromosomes 1 and 5, although this remains to be determined.

An additional chromosome of major interest in the FHH × BN consomic panel is chromosome 13, because substitution of BN chromosome 13 into the SS genetic background not only ameliorates the salt-induced increase in blood pressure in the SS rat (9) but also protects against salt-induced renal damage (9) and restores the impaired relaxation of resistance arteries that is present even in SS rats fed a low-salt diet (13, 14). As noted above, PRA in the FHH rat is 10 times higher than PRA in the BN rat (40) and is even higher than that in the SS rat, which is exposed to chronically low PRA during low-salt diet because of an inability to regulate its renin gene normally (9). While it is well known that supraphysiological levels of ANG II lead to vascular oxidant stress and endothelial dysfunction, exposure to chronically low levels of ANG II also lead to an increase in vascular superoxide levels, endothelial dysfunction, and impaired vascular relaxation in SS rats fed a low-salt diet (13, 14), presumably due to downregulation of antioxidant defense mechanisms. In the case of the SS rat, vascular oxidant stress is ameliorated and normal vascular relaxation mechanisms are restored by introgression of BN chromosome 13, which contains the normally functioning renin allele from the BN rat (13, 14). In contrast to the dramatic effects of BN chromosome 13 on vascular activity in the SS rat (13, 14), introgression of BN chromosome 13 into the FHH rat had much less effect on vascular reactivity in the FHH × BN consomic panel, aside from salt-induced increases in PE sensitivity (males) and slow slope of PE-induced contraction (females) (Tables 1 and 2) and a sex-specific reduction in vascular Ach sensitivity in FHH-13BN rats fed a low-salt diet (Table 3). Taken together, these findings suggest that impaired vascular relaxation may be present in systemic resistance arteries of FHH rats fed a low- or normal-salt diet, and that the underlying basis of impaired vascular function in the FHH rat may be different from that in the SS rat. Because of the extremely high PRA in the FHH rat (40), any vascular dysfunction in this rodent model of hypertension may be related to elevated PRA and ANG II levels, suggesting that the FHH rat may be a valuable rodent model to investigate the genetic mechanisms of vascular dysfunction in high-renin forms of hypertension.

Of the other chromosomes, introgression of chromosomes 11 (6 phenotypes), 12 (5 phenotypes), 16 (5 phenotypes), 17 (6 phenotypes), and 18 (6 phenotypes) had the greatest impact on aortic ring reactivity in the FHH × BN consomic panel. However, relatively few of the studies reporting QTLs for different physiological and pathological phenotypes in the rat genome implicate chromosomes 11, 12, and 17 in blood pressure or vascular regulation. For example, two studies reported a QTL for Ca²⁺ signaling and platelet aggregation on chromosome 12 in rats derived from backcrosses of SHR and Fisher 344 rats (50, 51), and QTLs have been reported on chromosome 17 for blood pressure in an SS × Lewis cross (12, 23). Other studies suggest that QTLs on chromosomes 16 and 18 play a somewhat greater role in regulating blood pressure and vascular function than QTLs on chromosomes 11, 12, and 17. For example, QTLs have been reported on chromosome 16 for salt sensitivity of systolic blood pressure in an SHR × BN cross (1); for blood pressure in congeneric SS rats carrying Lewis alleles (45); for sex-specific renal disease in stroke-prone SHR (21); and for blood pressure and metabolic disease in a cross of Lyon hypertensive × Lyon normotensive rats (5). In the case of chromosome 18, QTLs have been reported for hypertension and salt sensitivity in an SHR × BN cross (27) and for blood pressure in an F₂ hybrid cross of SHR and normotensive BB/OK spontaneously diabetic rats (28).

The existence of multiple vascular phenotypes that are altered by chromosomal substitution in the FHH × BN consomic panel in the absence of extensively reported QTLs for blood pressure regulation suggests that FHH rats may exhibit substantial alterations in arterial vascular reactivity that may become manifest even prior to (or independent of) the elevation in arterial blood pressure in this genetic model of hypertension, and that the alterations in arterial vascular reactivity in FHH (and their underlying mechanisms) may differ from those in many other models of hypertension. If alterations in vascular reactivity (especially impaired vascular relaxation in response to endothelium-dependent vasodilator stimuli such as Ach and reduced P2) are present in systemic resistance arteries of FHH rats, this experimental model could be a valuable tool for investigating cardiovascular disease in humans, in whom endothelial dysfunction is often a harbinger of adverse cardiovascular events (including death), even in the absence of an elevated blood pressure (71).

Strain-Specific Differences in Effect of Different Chromosomal Substitutions on Aortic Vascular Reactivity

The most remarkable finding in the present study is that the chromosomes that appear to be involved in the modulation of aortic ring reactivity are highly strain specific. As summarized in Table 5, consomic strains showing a significant increase or decrease in aortic reactivity to vasoactive substances compared with the FHH parental strain in the FHH × BN panel (see results) are quite different from those showing altered responses of the aorta to vasoactive stimuli in the corresponding consomic strains from the SS × BN panel (33, 34).

Despite the fact that substitution of the vast majority of BN chromosomes into either the SS or the FHH genetic background produced some effect on one or more of the vascular phenotypes assessed in the PGA studies, there was remarkably little overlap in the effect of a specific BN chromosome on a specific vascular phenotype in the two recipient strains (FHH vs. SS). For example, genes on chromosomes 1, 15, 16, 18, and 20 modify l-NAME-induced hypertension and proteinuria in the FHH rat (40). However, the relative effect of these chromosomal substitutions (and others) on salt-induced changes in vascular reactivity or sex-specific differences in vascular phenotypes varied substantially between chromosomes.

Substitution of BN chromosome 16 into the FHH background had comparatively few effects on vascular responses in FHH-16BN rats but affected 15 different vascular reactivity phenotypes in the SS × BN panel (33, 34), where this chro-
mosome appeared to be especially important in mediating changes in vascular reactivity in SS-16\textsuperscript{BN} consomic rats fed a high-salt diet versus low-salt control rats [e.g., decreased sensitivity to ACh and PE in males, increased maximum relaxation to ACh in females, increased SNP sensitivity in males and females (33), and a reduced sensitivity to 10% O\textsubscript{2} and 5% O\textsubscript{2} in females (34)]. SS-16\textsuperscript{BN} consomic rats also showed sex-specific differences in aortic reactivity on low-salt diet, including decreased PE sensitivity, increased maximum force of contraction, reduced ACh sensitivity, and increases in SNP sensitivity and maximum relaxation to ACh (34).

Introgression of chromosome 2, which has a QTL for mean arterial pressure in SS rats (53) and has potentially beneficial effects on renal disease in female consomic rats from the FHH × BN cross (40), affected only two vascular phenotypes in the FHH × BN consomic panel (Tables 1 and 4) but had multiple effects on vascular reactivity (12 different phenotypes in the various groups) in consomic rats from the SS × BN panel (33, 34). In a similar fashion, introgression of BN chromosome 8 into the FHH background, which has potentially beneficial effects on renal disease in the FHH × BN consomic panel (40), had no effect on aortic ring reactivity in any of the comparisons in the FHH × BN consomic panel but had multiple effects (15 different phenotypes) on vascular reactivity in the SS × BN consomic panel (33, 34).

Comparison of Salt-Dependent and Sex-Specific Changes in Vascular Phenotypes in FHH × BN and SS × BN Consomic Panels

When vascular reactivity effects are compared over both consomic panels, the lack of similarity of the effects of chromosomal substitution on salt-induced phenotypic changes or sex-specific differences in phenotypes (cf. Tables 1–4 vs. Refs. 33, 34) in consomic rats from the two crosses was striking. First, chromosomal substitution affected more of these phenotypes in the SS × BN panel (166 total phenotypes over all the chromosomal substitutions) than in the FHH × BN panel (91 total phenotypes over all the chromosomal substitutions). Over the entire set of comparisons, substituting the same BN chromosome into the SS versus the FHH background significantly affected the same vascular phenotype only 7.0% of the time (18 of 257 of total affected phenotypes). For phenotypes directly related to vessel responses to vasoconstrictors (PE) and vasodilator stimuli (ACh, nitroprusside, reduced P\textsubscript{O\textsubscript{2}}), substitution of the same BN chromosome into the SS versus the FHH background significantly affected the same vascular phenotype only 7.0% of the time (18 of 257 of total affected phenotypes). For phenotypes directly related to vessel responses to vasoconstrictors (PE) and vasodilator stimuli (ACh, nitroprusside, reduced P\textsubscript{O\textsubscript{2}}), substitution of the same BN chromosome into the SS versus the FHH genetic background affected the same phenotype in only 4.6% of the cases (9 of 194 total vascular reactivity phenotypes where significant changes occurred—65 in the FHH × BN panel and 129 in the SS × BN panel). Of these affected phenotypes, 3.1% (6 of 194 vascular reactivity phenotypes) changed in the same direction in the SS × BN and FHH × BN panels, and 1.5% (3 of 194 phenotypes) changed in the opposite direction in the two consomic panels.

Summary and Perspectives

The aortic ring preparation is used extensively to identify factors affecting vascular reactivity in health and disease and is ideally suited to high-throughput analysis of the role of different genetic influences in regulating vascular reactivity. The present study shows that substitution of several individual chromosomes from the BN rat into the FHH genetic background has a significant effect on aortic ring reactivity in the consomic rats from the FHH × BN cross, while others do not. Of the multiple phenotypes evaluated in the present study, the ones that are likely of the greatest interest in evaluating the genomic basis of vascular regulation in the FHH model of
hypotheses are likely to be the response of the aorta to PE (a classic adrenergic vasoconstrictor agonist widely used in studies of vasoconstrictor sensitivity in different experimental models of hypertension) and the sensitivity and maximum amplitude of the aorta in response to the widely used endothelium-dependent vasoconstrictor ACh. Of particular interest among stressors and phenotypes included in the present study is the effect of elevated dietary salt intake, which has been shown to have dramatic effects on vascular relaxation, vascular oxidant stress, and endothelial function, independent of changes in arterial blood pressure (38, 69). An additional area of interest is the role of specific genes and chromosomes in contributing to sex-specific differences among strains.

There are several important observations in the present study: 1) the chromosomal substitutions that affect aortic ring reactivity are often different from those affecting blood pressure and/or kidney disease; 2) sex-specific differences exist in the effect of various chromosome substitutions; and 3) multiple chromosomes are associated with the effects of dietary salt intake on aortic ring reactivity. Finally, when the FHH × BN (present study) and SS × BN (33, 34) consomic panels are compared, the chromosomes that affect different aortic reactivity phenotypes as a function of strain, sex, or dietary salt intake are strikingly different in the two consomic panels, suggesting that the genetic background is of great importance when studying the function of a specific gene or the development of a disease.

Taken together, the findings of the present study and those of previously reported studies (33, 34) suggest that genetic determinants of hypertension and renal disease in FHH rats may be independent of those affecting vascular regulation in FHH rats but may participate in vascular regulation in the SS rat. These findings also suggest that different genes can affect vascular regulation independent of blood pressure, and that different genes (and different chromosomes) are likely to contribute to vascular dysregulation in different forms of hypertension, for example, salt-sensitive hypertension, high- and low-renin hypertension, and essential hypertension.

In the present study, all of the changes in measured phenotypes could be related to the addition of the BN chromosome to the FHH genetic background and/or to the absence of a single FHH chromosome. However, some caveats are in order regarding the findings of consomic strategies to evaluate vascular reactivity in aortic rings. For example, gene-to-gene interactions, the importance of the genetic background of the recipient strain, and epistasis must be taken into account when evaluating these results. It is also important to note that different genes are likely involved in the effects of specific chromosomal substitutions on different phenotypes within the same consomic panel (or in different consomic panels). Finally, there may be regional differences in the mechanisms regulating vascular reactivity in aortic rings versus arterioles and resistance arteries and in arterioles and resistance arteries from different vascular beds. Nonetheless, Monti and coworkers (43) noted that the use of chromosomal substitution strains provides the advantage of systematically and more fully accounting for quantitative phenotype variation due to epistasis. Other authors (8) have stressed that consomic panels not only provide a greater power for initial mapping of phenotypic traits and QTLs but also allow even weaker effects to be identified with many fewer animals than required for segregating crosses and other mapping methods. Overall, high-throughput studies of changes in aortic reactivity resulting from substitution of individual chromosomes in consomic rats resulting from the FHH × BN cross can 1) provide initial valuable insight into the role of individual chromosomes in affecting vascular reactivity in the FHH genetic rat model of hypertension; 2) provide clues toward identifying the role of specific chromosomes in mediating altered vascular reactivity in the FHH model of hypertension versus other models, e.g., the Dahl SS rat model of salt-sensitive hypertension in humans (10, 11); and 3) pave the way for more precise identification of the genetic factors affecting vascular reactivity by expediting the development of narrowed congeneric strains (41) incorporating smaller segments of individual BN chromosomes into the FHH genetic background.

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DISCLOSURES

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