Original Article

Hemodynamic Characterization of Recombinant Inbred Strains: Twenty Years Later

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Recombinant inbred (RI) strains (Prague HXB/BXH set) represent a unique model that allows for permanent summation of genetic and physiological information as well as the study of age-dependent changes in phenotypes and/or gene regulation. This study compared blood pressure (BP) measured in adult animals of RI strains by radiotelemetry with BP values obtained in conscious rats of comparable age subjected to short-term carotid catheterization or with those obtained by direct carotid puncture under ether anesthesia (almost 20 years ago). After radiotelemetry recording, the contribution of major vasoactive systems to BP maintenance was studied by consecutive inhibition of the renin-angiotensin system (RAS), sympathetic nervous system (SNS), and nitric oxide synthase. We found highly significant interrelationships among baseline BP values obtained by radiotelemetry, carotid catheterization, or carotid puncture. This indicates considerable stability of RI strains over the course of their long existence, and confirms the reliability of BP values used for genetic studies performed in the past. Subsequent analysis of vasoactive system participation revealed the importance of SNS for the maintenance of BP, as determined by either radiotelemetry or catheterization. The BP of catheterized rats also correlated closely with acute captopril-induced BP changes, but this was not the case for rats measured by radiotelemetry. NO-dependent vasodilatation matched the BP effects of SNS and RAS in both measuring conditions. Residual BP (recorded at sodium nitroprusside–induced dilatation of resistance vessels) was also responsible for a significant portion of the BP variation in RI strains. Our study confirms the validity of RI strains for the further genetic and physiological research of hypertension. (*Hypertens Res* 2008; 31: 1659–1668)

***Key Words*: spontaneously hypertensive rat, radiotelemetry, blood pressure, organ weights, vasoactive systems**

Introduction

For more than 150 years, rats have been used as a model for a variety of experimental research studies (*1*). The first step toward solving the problems associated with the use of rats for genetic research was accomplished by the development of numerous inbred strains, often characterized by various abnormalities. These strains could be mated to produce F_1 generations and, finally, F_2 hybrids. Each F_2 hybrid individual carries a unique combination of genes due to independent segregation of maternal and paternal chromosomes and recombination between homologous chromosomes during gametogenesis in the F_1 generation. These F_2 hybrids represent unique gene combinations so that there is no possibility of repeating the experiment with the same combination of genes.

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Therefore, the set of recombinant inbred (RI) strains was developed from two widely divergent inbred strains, spontaneously hypertensive rats (SHR/Ola) and normotensive Brown-Norway rats (BN-Lx/Cub) (*2*). Brother-sister matings of randomly selected pairs of F_2 animals for more than 20 generations fixed the segregated genes of the F_2 hybrids. Thus, a series of more than 30 RI strains can be regarded as a reproducible F_2 generation. This is a great advantage of RI strains in comparison with F_2 hybrids because genotype and numerous phenotypes are already known, and thus one needs only to test new traits (*2*). RI strains represent a unique model that allows for permanent summation of genetic and physiological information as well as the study of age-dependent changes in phenotypes and/or gene regulation (*3*). At present, two reciprocal crosses are available, 21 HXB/Ipcv and 10 BXH/Cub (*4*), the genetic map of which contains more than 1,000 markers (*5*) and over 80 phenotypes have already been determined (http://www.genetwork.org/).

Blood pressure (BP) is a quantitative trait, the level of which depends on genetic and environmental factors. Recently, almost 200 quantitative trait loci (QTLs) associated with BP have been reported in different rat models, including spontaneously hypertensive rats (SHR) (Rat Genome Database at http://www.rgd.mcw.edu/). Genetic analysis of RI strains revealed QTLs for BP regulation on chromosomes 1, 2, 4, 19, and 20 (*4*). However, BP is a complex phenotype, and even small inaccuracies in its determination could considerably influence linkage results. Three major techniques are currently used for BP measurement, 1) tail-cuff plethysmography, 2) the fluid-filled catheter technique, and 3) long-term radiotelemetric measurements, with transmitters implanted into the abdominal cavity and connected to the abdominal aorta. This reliable method for monitoring physiological functions in awake and freely moving small laboratory animals (*6*) was introduced to hypertension research in order to measure cardiovascular parameters without the stress artifacts associated with the other methods (*7–9*). Almost 20 years ago, the values for systolic, diastolic, and mean arterial pressure were determined in RI strains and their progenitors by direct puncture of the carotid artery under light ether anesthesia (*2*), which is another technique for the simple, direct determination of BP.

We recently demonstrated that the altered balance between enhanced sympathetic vasoconstriction and relatively deficient NO-dependent vasodilatation could lead to high BP in genetic (*10*) as well as salt hypertension (*11*). It was demonstrated in both studies that basal BP in F_2 hybrids was positively associated with BP change induced by blockade of the SNS by pentolinium, and with residual BP.

The aim of the present study was to determine BP in RI strains by radiotelemetry under basal conditions and to compare the results not only with the BP of conscious catheterized animals, but also with original BP values (*2*) measured by direct carotid puncture 20 years ago. The balance of major vasoactive systems was measured in conscious animals at the end of the experiment by consecutive inhibition of the renin-angiotensin system (RAS), sympathetic nervous system (SNS), and nitric oxide synthase (NOS) (*10*, *12*, *13*).

Methods

Experimental Animals

RI strains were developed from two reciprocal crosses between progenitor strains BN-Lx/Cub (denoted as "B") and SHR/OlaIpcv (denoted as "H") (*2*, *14–16*). This set of RI strains, referred to as the HXB/BXH set, represents a powerful tool for association studies and linkage analyses in the rat. All rats used for the present experiments were obtained from the original colonies (HXB/Ipcv strains: Institute of Physiology AS CR, Prague, Czech Republic; BXH/Cub: Institute of Biology and Medical Genetics, Faculty of Medicine, Charles University, Prague, Czech Republic) in which these strains were developed. This allowed for a direct comparison of the present data with the original values reported in the same colonies 20 years ago (*2*).

All animals were maintained in temperature-controlled conditions (23±1°C) and a 12-h light/dark cycle (7 AM to 7 PM), fed a pellet diet (ST-1; Velaz, Prague, Czech Republic), and given tap water ad libitum. Thirty-one RI strains and their progenitors were used throughout this study, in which 6–8 animals were measured for each of the RI strains. The experiments were approved by the Ethical Committee of the Institute of Physiology AS CR, and conformed to the "European Convention on Animal Protection" and "Guidelines on Research Animal Use."

BP and Heart Rate Monitoring: Experimental Protocol

The Dataquest IV radiotelemetry system (Data Sciences International, St Paul, USA) was used for measurement of systolic, diastolic, and mean arterial pressure and heart rate. Transmitters (TA11PA) were implanted in 12-week-old males after calibration as recommended by the supplier. Briefly, the rats were anesthetized with ketamine (100 mg/kg) and xylazine (5 mg/kg), a midline abdominal incision was made, and abdominal aorta was exposed. The catheter of the transmitter was inserted rostrally into the aorta through a small hole close to the bifurcation and covered with a piece of nitrocellulose sheet to minimize the bleeding. The body of the transmitter was sutured to the inside of the abdominal muscle wall, while muscle and skin incisions were closed with sutures. Rats were housed individually in standard cages, which were placed over the receivers that were connected to the personal computer for data acquisition. The animals were allowed to recover for 10 d after the surgery. Thereafter, BP signals were sampled for 10 s every 10 min and the average values for systolic, mean arterial, and diastolic pressure as well as heart rate were stored in the personal computer. Basal BP and heart rate

Table 1. Old Values (Direct Carotid Puncture (*2*); Organ Weights (*21*)) and New Values (Carotid Catheterization: This Study) for MAP as Well as Relative Heart (HW/BW) and Kidney (KW/BW) Weights in Individual RI Strains and Their Progenitors (Animals Aged 18 Weeks)

	Old data			New data		
	MAP (mmHg)	HW/BW (mg/100 g)	KW/BW (mg/100 g)	MAP (mmHg)	HW/BW (mg/100 g)	KW/BW (mg/100 g)
SHR	181.7±3.5	322±3	636±6	178.2±4.6	346±4	776±13
BN-Lx	120.7±4.7	232±8	469±7	109.6±1.5	267±10	598±19
RI 1	129.7±3.7	275±4	464±5	134.6±2.2	295±5	703±37
RI 2	143.6±5.4	274±10	626±59	137.8±4.3	315±5	684±13
RI 3	140.0±4.7	261±6	596±13	126.1±4.6	271±5	648±9
RI 4	159.0±7.0	330±7	633±4	147.2±3.3	408±18	728±9
RI 5	134.0±0.6	299±19	600±16	138.1±4.0	306±8	665±24
RI 7	120.3±4.5	272±16	534±20	130.1±5.7	349±51	658±36
RI 10	142.2±5.5	290±4	589±8	131.5±1.8	302±7	678±13
RI 13	132.0±1.9	289±2	592±8	135.9±4.0	315±10	680±28
RI 15	119.0±3.9	276±4	741±10	125.2±2.6	265±6	750±21
RI 17	152.4±4.0	297±6	509±11	145.6±4.3	308±4	543±15
RI 18	123.6±3.5	290±8	500±14	130.8±2.8	295±3	690±17
RI 20	121.8±3.4	247±5	527±14	117.3±5.3	245±4	565±11
RI 21	154.5±5.9	286±7	553±29	136.3±4.0	308±5	648±17
RI 22	122.6±2.7	255±14	556±9	122.4±4.1	247±3	577±9
RI 23	141.4±5.3	262±8	559±8	127.9±3.2	284±17	633±13
RI 24	123.3±8.3	290±18	609±8	122.1±1.9	426±35	727±17
RI 25	121.3±7.2	288±2	598±2	131.5±4.2	317±12	721±22
RI 26	139.4±6.5	309±12	599±21	124.6±2.4	263±4	644±9
RI 27	123.7±9.7	234±7	528±10	117.0±2.4	255±19	581±35
RI 29	127.2±5.4	285±7	578±9	119.8±2.3	301±5	638±28
RI 31	129.0±1.0	302±7	546±7	115.4±4.8	285±3	643±11
RI 2c	126.6±2.5	291±9	608±5	116.0±2.7	306±10	732±14
RI 3c	130.5±2.6	264±6	645±15	121.1±2.7	268±4	659±15
RI 5c	137.5±3.9	263±6	537±8	126.5±2.5	303±7	681±27
RI 6c	131.0±4.2	294±8	628±16	125.3±3.0	272±4	699±12
RI 8c	114.0±2.1	308±3	627±14	123.1±2.8	337±23	728±17
RI 9c	138.0±3.4	283±8	553±12	123.3±4.2	278±7	636±12
RI 10c	124.8±5.2	257±4	513±9	116.7±2.0	296±4	732±26
RI 11c	139.8±5.8	278±3	615±16	127.3±3.0	274±11	676±21
RI 12c	144.9±7.8	284±6	483±7	117.7±5.2	312±8	687±22
RI 13c	142.3±4.4	307±5	653±3	132.5±3.9	320±5	718±17
F ratio	4.80*	4.19*	9.34*	5.53*	8.49*	8.12*
$Q^{\#}$	24.9	50.3	100.1	18.5	75.6	98.4

Data are means±SEM. Six to eight animals were measured in each RI strain. df_1/df_2: 30/138 (old data), 30/177 (new data). *$p<0.0001$. $^{\#}Q$ interval (at the level of $p<0.05$). SHR, spontaneously hypertensive rats; BN-Lx, normotensive Brown-Norway rats; MAP, mean arterial pressure; RI, recombinant inbred.

values were monitored for 14 d. The BP values for each RI or progenitor strain were calculated by averaging all telemetry recordings starting 1 week after stabilization of the measurement. Mean nighttime BP values representing the dark-period segment (7 PM to 7 AM) of seven consecutive days were calculated for each animal and then averaged across all animals of the same strain. Apparent outlier values were removed prior to the calculations. We also calculated daytime BP values (7 AM to 7 PM), which were used for the correlations with BP changes induced during consecutive blockade of particular vasoactive systems (see below).

At the end of the experiment, the balance of vasoactive systems was studied before termination of the experiment (see below). Finally, after neck dislocation, heart and kidney

weight was determined and a piece of the tail was stored at −80°C for later DNA studies.

Vasoactive Balance Studies

On the day before BP determination, polyethylene catheters were inserted into the left carotid artery and jugular vein and exteriorized in the interscapular region. BP was recorded 24 h later as described previously (*10*, *17*). Briefly, the animals were connected to Statham pressure transducers and a multichannel PowerLab (ADInstruments, Castle Hill, Australia) recording system. The system was calibrated before each recording and validated carefully with regard to signal dampening in the catheters. BP was always recorded in four animals simultaneously between 8 AM and 12 AM. Baseline BP values were monitored in conscious animals for 30 min. Thereafter, an intravenous bolus of captopril (Sigma, St. Louis, USA; 10 mg/kg b.w.) was injected to inhibit RAS. Ten minutes later, ganglion blockade was induced by pentolinium (Sigma; 5 mg/kg b.w.). After a rapid BP fall and its transient stabilization for about 5 min, N^G-nitro-L-arginine-methyl ester (L-NAME) (Sigma; 30 mg/kg b.w.) was given to inhibit NOS. The long-term BP increase after L-NAME administration was monitored for 20 min until stabilization, at which time sodium nitroprusside (SNP, 20 μg/kg b.w.) was injected to estimate the residual BP at full vasodilatation. All drugs (injected in a volume of 1 mL/kg) were given in doses that elicit maximal BP effects lasting considerably longer than the duration of our vasoactive system test.

Statistical Analysis

Data are expressed as means±SEM. Statistical differences were evaluated by the Student's *t*-test (in the case of a simple comparison between progenitors) or by one-way ANOVA followed by the post-hoc *Q* test (in the case of multiple comparisons within our set of RI strains) (*18*). Repeated measures one-way analysis of variance was used to test the effects of acute pharmacological interventions in the vasoactive balance studies performed in RI strains. Correlation analysis was used to evaluate the relationships between particular BP data obtained by the individual methods for BP determination as well as between BP and other phenotypes. Oldham transformation of the data (*19*) was used to avoid spurious associations between drug-induced BP changes and basal BP values. BP changes were therefore correlated with the mean of pre- and post-treatment BP values. A value of $p<0.05$ was considered as indicative of a significant relationship.

Results

New and Old Values of Directly Measured BP and Relative Organ Weight

Table 1 summarizes the old data (original values obtained by carotid puncture: Pravenec *et al*. (*2*)) and new data (carotid catheterization: this study) for mean arterial BP (MAP) and relative organ weights in 31 RI strains and their progenitors. One-way analysis of variance confirmed the presence of highly significant differences within this set of RI strains. It is evident from the comparison of new and old values that BP determination in conscious catheterized rats yielded comparable BP data as the first BP measurement in RI strains performed using a different technique almost 20 years ago. This was indicated by the highly significant relationship

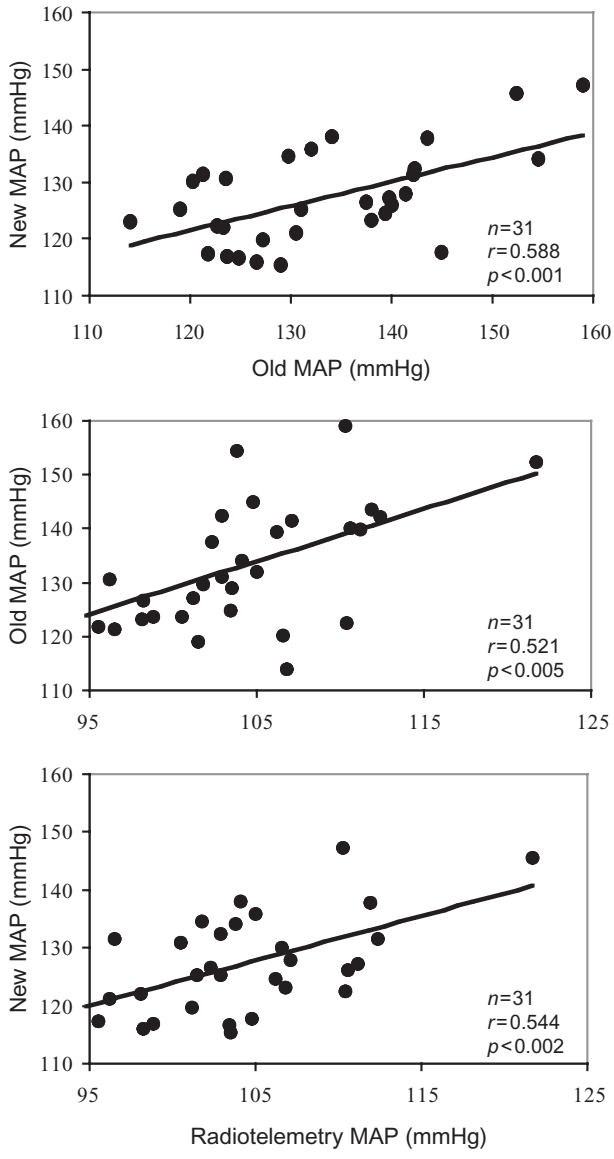

Fig. 1. *The relationships between old (direct puncture of carotid artery) and new (carotid catheterization) values for mean arterial pressure (MAP) (upper), between MAP values obtained by radiotelemetry and old MAP values (middle), and between radiotelemetry values and new MAP values (carotid catheterization) (lower) recorded in RI strains.*

Table 2. Baseline SBP, MAP, DBP, PP, and HR as Well as MAP Change after Inhibition of RAS (Captopril), SNS (Pentolinium), and NO Synthase (L-NAME) in SHR and BN-Lx Progenitor Strains at 18 Weeks of Age

	SHR (n=9)	BN-Lx (n=10)
SBP (mmHg)	222.0±5.2*	126.9±2.4
DBP (mmHg)	145.8±4.1*	92.0±2.6
PP (mmHg)	76.2±1.8*	34.9±4.0
Baseline MAP (mmHg)	178.2±4.6*	109.6±1.5
Basal HR (beats/min)	328±6	340±7
$MAP_{captopril}$ decrease (mmHg)	11.6±4.4	8.7±0.9
$MAP_{pentolinium}$ decrease (mmHg)	70.7±6.0*	43.7±2.4
$MAP_{L\text{-}NAME}$ increase (mmHg)	98.9±4.5*	65.1±5.9
Residual MAP after SNP (mmHg)	67.4±2.1*	50.8±4.3
Vasodilator deficit (mmHg)	−26.8±4.7*	−6.9±2.7

Data are means±SEM. Residual MAP, blood pressure reached after SNP injection of rats with combined inhibition of RAS, SNS, and NOS; vasodilator deficit, difference between residual MAP and MAP reached after inhibition of both vasoconstrictor systems (RAS and SNS). *p<0.01 compared with BN-Lx animals. SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; HR, heart rate; RAS, renin-angiotensin system; SNS, sympathetic nervous system; L-NAME, N^G-nitro-L-arginine-methyl ester; SHR, spontaneously hypertensive rats; BN-Lx, normotensive Brown-Norway rats; SNP, sodium nitroprusside.

(p<0.001) between old (direct puncture of carotid artery) and new (carotid catheterization) values for mean arterial BP (Fig. 1, upper). It is important to note that there was a highly significant relationship between nighttime BP data (recorded by radiotelemetry) and both old BP data (Fig. 1, middle, p<0.005) and new BP data (Fig. 1, lower, p<0.002). However, BP data obtained by radiotelemetry were always lower than those yielded by carotid puncture or catheterization, suggesting that stress artifacts were minimized during radiotelemetric BP recording. This is in good agreement with the data obtained by radiotelemetry in a subsequent colony of Prague RI strains maintained in La Jolla (University of California, San Diego, USA) (*20*).

There was also a highly significant correlation between old (Kuneš *et al*. (*21*)) and new values for relative heart weight (r=0.547, n=31, p<0.002) as well as relative kidney weight (r=0.448, n=31, p<0.02). New MAP values correlated positively with relative heart weight (r=0.399, n=31, p<0.05), but no significant relationship was found between new MAP values and relative kidney weight (r=0.042, n=31, n.s.). Somewhat stronger correlations were observed when we examined the relationships between pulse pressure and relative organ weights (found in the present experiments) (heart: r=0.531, n=31, p<0.002; kidney: r=0.239, n=31, n.s.).

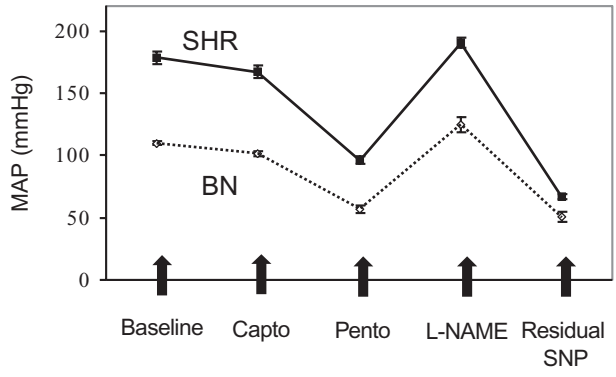

Fig. 2. *Baseline MAP in conscious rats of progenitor strains (SHR, BN-Lx) and MAP values recorded during consecutive inhibition of the renin-angiotensin system (captopril: Capto), sympathetic nervous system (pentolinium: Pento), and NO synthase (L-NAME) as well as after the administration of sodium nitroprusside (SNP, residual blood pressure).*

Vasoactive Balance

All examined components of BP were higher in adult SHR than in normotensive BN-Lx rats, but there was no difference in heart rate between both progenitor strains (Table 2). The difference in BP between SHR and BN-Lx was preserved, even after consecutive inhibition of RAS and SNS, as well as after the subsequent inhibition of NOS (Fig. 2). Captopril administration caused a similar mild decrease in MAP in the two strains, while the pentolinium-induced MAP fall was considerably greater in SHR than in BN-Lx animals. Similarly, the L-NAME–induced MAP increase was also enhanced in SHR (Table 2). The residual MAP recorded after SNP injection to rats subjected to combined inhibition of RAS, SNS, and NOS was also significantly elevated in SHR compared to BN-Lx rats (Fig. 2). Despite the much greater MAP change after L-NAME administration (indicating compensatory enhancement of NO production) in SHR, their vasodilator deficit was still significantly greater than that found in BN-Lx animals (Table 2).

Table 3 summarizes the MAP changes induced by the blockade of particular vasoactive systems, residual MAP values, and daytime MAP values determined by radiotelemetry in individual RI strains. One-way analysis of variance indicated that, within this set of RI strains, there were significant strain differences for all parameters analyzed. Repeated measures one-way analysis of variance revealed that each of the pharmacological interventions caused a highly significant (p<0.0001) MAP change in the whole set of RI strains. This analysis also disclosed that, on average, the vasopressor effects of RAS are responsible for 10 mmHg (8% of baseline MAP), whereas those of SNS account for 51 mmHg (40% of baseline MAP). Both vasopressor systems are well balanced by NO-dependent vasodilatation, which was equal to 73

Table 3. MAP Changes Induced in Conscious Chronically Cannulated Rats by Inhibition of Particular Vasoactive Systems (RAS, SNS and NOS) and Residual MAP Values in Individual RI Strains (Animals Aged 18 Weeks): Their Relationships with Daytime MAP Were Determined by Radiotelemetry

	$\Delta MAP_{captopril}$ (mmHg)	$\Delta MAP_{pentolinium}$ (mmHg)	$\Delta MAP_{L\text{-}NAME}$ (mmHg)	Residual MAP (mmHg)	Daytime MAP (mmHg)
RI 1	10.2±3.1 (5)	59.2±5.1	80.8±3.3	60.9±1.5	102.0±6.6 (5)
RI 2	10.9±2.4 (6)	54.0±4.5	81.0±4.7	68.9±5.7	109.9±4.4 (7)
RI 3	10.1±2.6 (6)	58.6±4.6	77.2±6.0	49.9±4.3	106.2±1.5 (6)
RI 4	11.2±3.2 (7)	63.7±3.3	67.2±4.7	63.4±1.6	105.6±1.7 (7)
RI 5	16.5±3.4 (6)	52.4±4.4	75.1±5.9	61.4±1.6	103.0±2.6 (7)
RI 7	11.7±2.2 (5)	46.4±5.7	76.5±6.8	66.1±0.8	101.8±4.3 (6)
RI 10	9.3±2.1 (5)	54.9±4.2	77.1±6.7	63.4±3.0	108.5±1.5 (7)
RI 13	12.4±2.3 (5)	60.2±2.1	91.1±2.0	64.0±1.4	100.9±1.6 (6)
RI 15	4.1±2.7 (8)	49.8±2.7	75.0±4.6	60.7±1.1	97.5±0.4 (8)
RI 17	10.6±1.7 (7)	59.0±4.4	93.0±8.7	72.1±4.1	120.6±0.9 (7)
RI 18	14.2±3.6 (7)	53.9±5.7	85.4±4.3	57.5±3.1	96.9±2.0 (7)
RI 20	6.7±2.5 (6)	54.7±6.6	61.5±9.1	53.1±1.3	93.0±1.3 (6)
RI 21	6.7±2.2 (7)	41.4±4.3	76.7±2.9	71.3±0.8	98.5±2.4 (7)
RI 22	2.3±1.3 (10)	55.3±3.7	68.4±5.8	61.2±5.3	102.8±1.3 (12)
RI 23	10.1±1.4 (6)	49.1±2.6	61.9±7.2	58.4±2.2	103.3±2.6 (6)
RI 24	7.0±0.7 (9)	52.6±2.0	52.2±5.9	59.8±1.4	95.6±4.0 (10)
RI 25	10.0±1.1 (8)	50.9±3.5	75.6±5.3	67.6±3.6	96.9±2.6 (8)
RI 26	10.8±0.9 (6)	54.0±2.0	73.3±3.0	59.5±1.5	99.4±1.1 (6)
RI 27	11.6±2.0 (6)	46.9±1.7	72.5±3.5	58.9±2.7	95.0±1.3 (6)
RI 29	9.5±2.1 (5)	46.5±4.3	56.4±4.6	59.0±2.9	98.8±2.6 (5)
RI 31	6.7±1.8 (5)	39.5±7.7	72.7±5.5	63.7±4.5	101.0±2.9 (6)
RI 2c	7.8±0.7 (12)	49.2±2.1	71.8±3.0	57.5±1.6	94.4±3.5 (12)
RI 3c	9.6±0.7 (7)	47.5±2.4	84.2±7.2	60.0±2.2	93.1±1.3 (8)
RI 5c	14.2±3.2 (8)	50.0±4.2	77.4±2.3	57.5±1.5	98.3±1.8 (8)
RI 6c	10.1±1.3 (6)	48.8±2.6	74.7±2.9	64.2±5.9	101.7±0.9 (6)
RI 8c	7.1±1.3 (5)	45.4±5.0	75.6±3.9	69.9±5.1	98.6±1.2 (7)
RI 9c	8.3±1.1 (6)	46.7±4.3	57.2±4.4	61.0±4.3	92.8±3.8 (6)
RI 10c	4.1±6.9 (5)	56.0±3.2	62.9±5.7	48.5±3.3	99.9±2.4 (5)
RI 11c	7.0±1.4 (5)	61.6±2.0	76.7±1.6	52.6±2.0	103.3±1.0 (7)
RI 12c	6.7±4.2 (5)	50.5±3.4	74.9±6.2	60.5±2.5	99.3±1.6 (7)
RI 13c	17.6±3.1 (6)	47.1±9.5	72.6±7.1	58.8±4.4	99.1±4.1 (6)
F ratio	2.265	1.677	2.970	2.707	4.617
df_1/df_2	30/177	30/177	30/177	30/177	30/186
p	<0.001	<0.025	<0.0001	<0.0001	<0.0001
$Q^{\#}$ (mmHg)	12.1	21.7	27.8	16.9	13.6
×daytime MAP	$r=-0.133$	$r=-0.463$	$r=0.434$	$r=0.345$	
p	n.s.	<0.01	<0.02	<0.06	

Data are means±SEM (*n*). $^{\#}Q$ interval (at the level of $p<0.05$). ΔMAP, blood pressure changes after inhibition of particular vasoactive systems; daytime MAP, basal MAP measured by radiotelemetry during the light segments (7 AM–7 PM) of seven consecutive days. MAP, mean arterial pressure; RAS, renin-angiotensin system; SNS, sympathetic nervous system; NOS, nitric oxide synthase; RI, recombinant inbred; L-NAME, N^G-nitro-L-arginine-methyl ester.

mmHg (58% of baseline MAP). The greater vasodilator impact of nitric oxide compared to the vasopressor effects of both RAS and SNS is fully compatible with the fact that most of the studied RI strains demonstrated BP values closer to the normotensive BN-Lx progenitors. Residual MAP (recorded after SNP injection) represented 48% of the baseline MAP.

In order to examine a possible differential effect of particular inhibitors on BP, we calculated the correlations of the above MAP changes with the baseline (pretreatment) MAP of individual RI strains. The baseline MAP of the RI strains was significantly related not only to captopril-induced and pentolinium-induced MAP changes, but also to L-NAME–induced

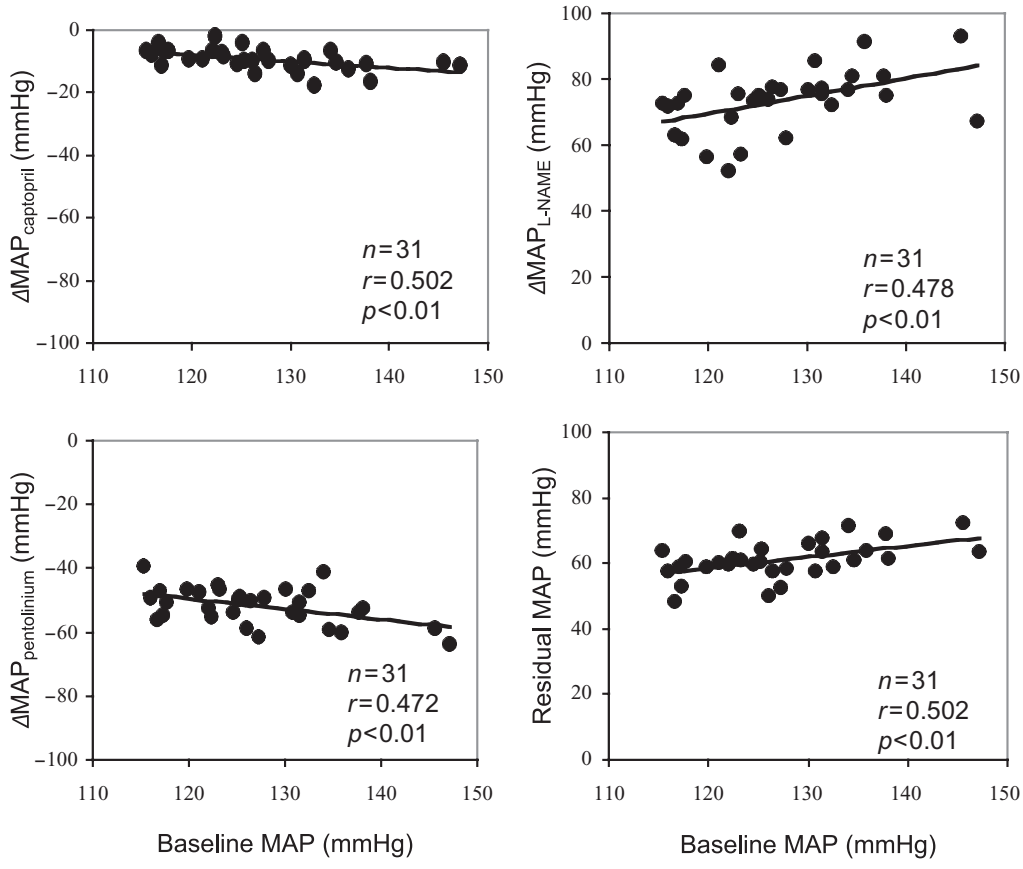

Fig. 3. *Relationships between the baseline MAP of catheterized rats and particular MAP components revealed by consecutive inhibition of various vasoactive systems in RI strains: MAP decrease after inhibition of RAS by captopril ($\Delta MAP_{captopril}$, upper left), MAP decrease after inhibition of SNS by pentolinium ($\Delta MAP_{pentolinium}$, lower left), MAP increase after inhibition of NOS by L-NAME ($\Delta MAP_{L\text{-}NAME}$, upper right), and residual MAP after sodium nitropruside injection (lower right). Regression lines represent the analysis of the means of 31 RI strains.*

MAP changes and residual MAP (Fig. 3). To avoid potential spurious associations of BP changes with baseline BP, we recalculated these three relationships using Oldham's approach in which BP changes are correlated with the mean of pre- and post-treatment BP values. Using this approach, we found a borderline significant ($p=0.07$) differential effect of captopril, but this was not the case for the differential effect of pentolinium. On the other hand, the differential effect of L-NAME was highly significant ($p<0.0001$), even after Oldham's transformation.

Detailed analysis of the contribution of particular vasoactive systems to BP maintenance in conscious chronically cannulated rats (Table 4) indicated not only absolute RAS hyperactivity, but also a tendency toward a relative hyperactivity of this vasopressor system in RI strains with elevated BP. On the other hand, SNS contribution to BP maintenance was characterized by an absolute hyperactivity of this vasoconstrictor system in RI strains with higher BP, but there were no signs of relative SNS hyperactivity in these RI strains (Table 4). It should be noted that a strong positive association of the NO-dependent component of BP regulation disappeared, or was even inverted, if BP changes elicited by acute L-NAME administration were taken as relative values (expressed in terms of pretreatment BP levels). This phenomenon suggests a relative NO deficiency in RI strains with higher BP because our data indicate that NO-dependent vasodilatation increases with progressively increasing BP, although its magnitude is not augmented enough to match enhanced RAS- and SNS-dependent vasoconstriction. Although residual MAP was highly significantly associated with baseline MAP in this set of RI strains, its augmentation also lagged behind the overall MAP increase. This is documented by the inversion of the strongly positive relationship of its absolute values to baseline MAP when the relative values for residual BP were used for the correlation (Table 4). Finally, it should be noted that the analysis based on individual animals yielded similar data as the same analysis using average values calculated in particular RI strains, although their statistical power was substantially greater (Table 4).

To evaluate the importance of selected vasoactive systems

Table 4. Relationships of Baseline MAP Values (Found in Conscious Chronically Cannulated Rats) to Absolute (Δ) or Relative (%Δ) MAP Changes Elicited by Inhibition of Particular Vasoactive Systems: a Comparison of Calculations Based upon Either Average RI Strain Values or Individual Animals

Average RI strain values (n=31)				
Baseline MAP×	Δcapto	Δpento	ΔL-NAME	Residual MAP
r	−0.502	−0.472	0.477	0.502
p	<0.002	<0.05	<0.05	<0.002
Baseline MAP×	%Δcapto	%Δpento	%ΔL-NAME	%Residual MAP
r	−0.349	0.137	−0.037	−0.228
p	<0.06	n.s.	n.s.	n.s.
Individual values (n=200)				
Baseline MAP×	Δcapto	Δpento	ΔL-NAME	Residual MAP
r	−0.378	−0.444	0.262	0.447
p	<0.0001	<0.0001	<0.001	<0.0001
Baseline MAP×	%Δcapto	%Δpento	%ΔL-NAME	%Residual MAP
r	−0.260	−0.003	−0.196	−0.274
p	<0.001	n.s.	<0.01	<0.001

MAP, mean arterial pressure; RI, recombinant inbred; capto, captopril; pento, pentolinium; L-NAME, N^G-nitro-L-arginine-methyl ester.

for BP maintenance in RI strains, we evaluated the relationship of BP changes induced by the blockade of particular vasoactive systems to baseline MAP values yielded by long-term radiotelemetric BP monitoring. Table 3 shows that both the sympathetic BP component and NO-dependent BP component (measured in conscious chronically cannulated rats) correlated significantly with daytime MAP values obtained by radiotelemetry. There was also a borderline association between daytime MAP values and residual MAP, but the RAS-dependent BP component had no significant relationship with daytime MAP (Table 3). Similar relationships were also revealed when we correlated the particular BP components with nighttime MAP values (sympathetic component: r=−0.453, n=31, p<0.02; NO-dependent component: r=0.400, n=31, p<0.05). These data indicate that SNS is important for BP maintenance in RI strains, especially if we consider that the radiotelemetry method is free of major stress artifacts. Again, NO-dependent vasodilatation surpassed the influence of both major vasopressor systems, which might be a possible explanation for the lower BP revealed by radiotelemetry compared to catheterization or direct puncture of the carotid artery.

Discussion

In 1989, we published the first analysis of BP in a set of RI strains developed from SHR and BN-Lx progenitors (*2*). BP was measured by direct puncture of the carotid artery under light ether anesthesia. The results of the present study revealed the accuracy of our BP determination in RI strains almost 20 years ago. This was confirmed not only by BP measurement in conscious animals through catheter inserted into the carotid artery, but also by radiotelemetry. Moreover, our demonstration of lower BP values obtained by radiotelemetry compared to those obtained by catheterization is in accordance with some studies (*22*, *23*) indicating that stress artifacts inherent to different methods for BP determination are lower when using a radiotelemetry system. Bazil *et al.* (*22*) demonstrated that tethering of SHR significantly increased BP and HR values for at least 48 h. On the other hand, BP determination in SHR by the tail-cuff method has many disadvantages because SHR are hyperresponsive to stressful stimuli (*24*). Therefore, use of the radiotelemetry device could be a big advantage in SHR and RI strains. However, a very good correlation between the radiotelemetry data and data from externalized catheters in the present study suggested that the use of chronically tethered rats also provides relevant BP data.

The present values obtained for relative organ weights are in good agreement with our previous study in RI strains (*21*). It is evident that relative heart weight (but not relative kidney weight) had a positive relationship with BP measured in conscious catheterized animals. This supports our former statement that genetic factors could play a predominant role in the determination of kidney weight, while heart weight should partially reflect the impact of BP level. We have previously demonstrated a significant relationship between newborn and adult relative kidney weight in RI strains (*3*). Several suggestive quantitative trait loci were detected for both newborn and adult relative kidney weights, indicating the usefulness of RI strains for age-dependent hypertension-related phenotypes, some of which were already abnormal before the development of high BP. Moreover, the relative heart weight in newborns of RI strains also correlated with this parameter in adult animals of the respective RI strains (*25*). A contemporary paper (*26*) shows significant associations between reduced fetal weight and both increased insulin concentration during the oral glucose tolerance test and increased serum and

hepatic triglycerides in adulthood.

Our previous studies on the vasoactive balance in F_2 hybrids indicated the importance of SNS for the maintenance of high BP in both genetic (*10*) and salt hypertension (*11*). It is important to note that, in the present study, the sympathetic BP component (pentolinium-induced MAP changes) correlated significantly with baseline BP values obtained by long-term radiotelemetry or in catheterized rats. A rather unexpected finding of this study in RI strains was the apparent ability of the vasodilator NO system (L-NAME–induced MAP changes) to counterbalance the vasopressor effects of both RAS and SNS in catheterized rats. Similarly, the NO system matched the BP effects of SNS very well in animals measured by radiotelemetry. This seems to be at variance with our earlier observations of a relative NO deficiency (lower NO-dependent vasodilatation compared to enhanced sympathetic vasoconstriction) in rats with genetic or salt hypertension (*10*, *11*, *13*). This discrepancy could be ascribed to strain differences, the greater age of the animals, or the absence of truly hypertensive rat strains in the present study. If we analyzed the present RI data at the level of individual animals, we observed a significant association ($r=0.189$, $n=200$, $p<0.01$) between baseline MAP and the ratio of MAP changes induced by captopril plus pentolinium to MAP changes elicited by L-NAME, indicating greater augmentation of vasoconstriction over NO-dependent vasodilatation in hypertensive rats. On the other hand, our findings on the important role of residual BP (reflecting geometric changes in the sodium nitroprusside–dilated resistance vasculature) for the observed BP elevation are in good agreement with similar findings made in salt hypertensive Dahl rats (*12*) and their F_2 hybrids (*11*), as well as F_2 hybrids derived from Lewis×hereditary hypertriglyceridemic rats (*10*).

Although the aim of this paper was not to reevaluate all previous findings in RI strains, namely the reported associations of BP and organ weights with genes of interest, on the basis of newly determined data, we were pleased to confirm several important associations of *Scnn1b*, *Ren*, *Hsp70*, or *Tnfa* genes with new direct and/or radiotelemetric BP values (data to be published).

It is evident that both physiological and genetic approaches must be used for determination of the true role of particular genes in the pathogenesis of hypertension, as documented by two recent papers on the catechol-*O*-methyltransferase gene in salt hypertensive Dahl rats (*27*, *28*).

In conclusion, analysis of the altered balance of principle vasoactive systems in RI strains indicated a major importance of both sympathetic nervous system and residual BP (recorded at full sodium nitroprusside–induced vasodilatation of resistance vessels) for setting the BP level in particular strains of this unique genetic model. Our present results obtained using HXB/BXH RI strains demonstrate the usefulness of this set not only for genetic studies, but also for intermediate phenotype "hunting." The main contribution of our study is the demonstration of considerable agreement between BP values obtained using three different techniques. This was even true for data obtained almost 20 years ago. However, one should keep in mind that each technique for BP measurement could characterize BP in a different way, and thus raise the possibility of searching for different genes involved in different steps of BP regulation.

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