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IMPLICATION OF CHROMOSOME 13 ON HYPERTENSION AND ASSOCIATED DISORDERS IN LYON HYPERTENSIVE (LH) RATS

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Abstract

Hypertension and associated disorders are major risk factors for cardiovascular disease. The Lyon Hypertensive rat (LH) is a genetically hypertensive strain that exhibits spontaneous and salt-sensitive hypertension, exaggerated proteinuria, high body weight, hyperlipidemia and elevated insulin-to-glucose ratio. Previous genetic mapping identified Quantitative Trait Loci (QTL) influencing blood pressure on chromosome 13 (RNO13) in several models of hypertension. To study the effects of a single chromosome on the mapped traits, we generated consomic strains by substituting LH RNO13 with that of the normotensive Brown Norway (BN) strain (LH-13^{BN}) and reciprocal consomics by substituting a BN RNO13 with that of LH (BN-13^{LH}). These reciprocal consomic strains, as well as the two parental strains were characterized for blood pressure, metabolic and morphological parameters. Compared to LH parents, LH-13^{BN} rats showed decreased mean blood pressure (up to −24 mmHg on 2%NaCl in the drinking water), urine proteins and lipids, and increased body weight. Differences between BN-13^{LH} and BN rats are much smaller than those observed between LH-13^{BN} and LH rats, demonstrating the effects of the highly resistant BN genome background. Plasma renin activity is not affected by the substitution of RNO13, despite the significant blood pressure differences.

The present work demonstrates that RNO13 is a determinant of BP, proteinuria, and plasma lipids in the LH rat. The distinct phenotypic differences between the consomic LH-13^{BN} and the LH make it a powerful model to determine genes and pathways leading to these risk factors for cardiovascular and renal disease.

Keywords

rat; consomic; hypertension

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INTRODUCTION

Hypertension is an important public health problem affecting about 65 million individuals in the United States alone [1]. Essential hypertension is traditionally viewed as a consequence of interactions between environmental and genetic factors (multifactorial). Despite numerous attempts, the majority of these genetic determinants remain to be identified [2]. One way to avoid some of the complications associated with genetic research in humans (heterogeneity of population, variability of environment), is to use animals models. Because the coding regions of the rat genome sequence are on average > 85% identical to that of man, there is a clear probability that the function of some genes identified in rats can be translated directly to human or, failing this, can be used as "indicators" [3].

The Lyon Hypertensive (LH/Mav) rat strain [4], initially selected for spontaneous hypertension, shares many features with the human metabolic syndrome [5,6,7,8,9,10,11,12]. Indeed, this genetically hypertensive strain also suffers salt-sensitive hypertension [13], altered renal functions with an exaggerated proteinuria [14], an excessive body weight together with increased plasma lipids and insulin-to-glucose ratio [15].

To find the genetic causes of multifactorial diseases like hypertension and associated disorders, our approach began with the identification of Quantitative Trait Loci (QTLs) which are statistically linked to the blood pressure (BP) level or to any other related phenotype. Since linkage analysis, in Dahl Salt Sensitive rats (SS) [16], Spontaneous Hypertensive Rats (SHR) [17,18,19,20] as well as in LH rats [21,22] suggested that a QTL(s) influencing blood pressure exist on rat chromosome 13 (RNO13), particularly in or near the renin gene, we sought to study the role of RNO13 in the LH rat. Therefore, we started the development of reciprocal consomic rat strains, i.e. animals which differ from the parental strains by a whole chromosome (here RNO13) using marker assisted selection [23]. We choose as normotensive control strain, the Brown Norway (BN/NHsdMcwi) rat, which is genetically divergent from the LH. LH-13^{BN} is a LH rat in which RNO13 has been fully substituted by a BN RNO13; conversely, BN-13^{LH} is a BN rat with a RNO13 from LH rats. Using a highly divergent strain was advantageous for two reasons: 1. it provided polymorphic markers spanning the chromosome to ensure complete chromosome substitution and 2. it allows us to uncover the maximum phenotypic differences due to genetic variation, including those not captured by the previous cross between LH and LN, strains with high genetic similarity. In order to determine the functional importance of RNO13, male LH-13^{BN}, BN-13^{LH} as well as the parental LH and BN strains, were characterized for not only blood pressure but also several additional phenotypes typical of LH rats, as additional significant traits could be unmasked with the substitution of the BN chromosome.

METHODS

Generation of consomic rats

The development of the consomic strains in this study involved the reciprocal transfer of the RNO13, from BN (BN/NHsdMcwi) to the genetic background of LH (LH/Mav), and from LH to the genetic background of BN. This required the production of an F1 generation by crossing a male LH with a female BN. The male F1 hybrids obtained were then mated to the desired recipient strain (LH or BN). After every backcross, DNA was extracted from tail tips and genotyped with markers evenly distributed on RNO13 at a 5 cM resolution (D13Mgh13, D13Rat39, D13Rat91, D13Mit 4, D13Mgh3, D13Rat 37, D13Rat85, D13Rat33, and D13Rat88) and 146 markers spanning the remaining genome at a ~10cM resolution as previously described [24]. Polymorphic markers were selected using the Genome Scanner Tool available at the Rat Genome Database (RGD; http://www.rgd.mcw.edu). Male rats,

heterozygous for the entire chromosome, were selected for the subsequent generation of breeding. The backcrosses were followed until obtaining animals heterozygous for the full length of RNO13 and homozygous LH or BN for the rest of the genome. These animals were then intercrossed to fix the donor RNO13 as BN or LH and to obtain founders for the reciprocal consomic strains. The reciprocal consomic lines (LH-13^{BN} and BN-13^{LH}) were then maintained by brother-sister mating.

Animals

The animals (LH, LH-13^{BN}, BN-13^{BN} and BN) were housed in controlled conditions (temperature 21±1°C, humidity 60±10%, lighting 8AM to 8PM), and received a standard rat chow containing 0.3% sodium chloride (Elevage AO3, SAFE, Augy, France) and tap water ad libitum. The protocols leading to these results followed our institutional guidelines which have been approved by the veterinary services of the prefecture.

Protocol

This study is a component of a larger project to characterize consomic strains for several chromosomes derived from LH and BN rats.

Because of technical considerations, the study of all the consomic strains, as well as the parental strains, was performed simultaneously. Consequently, the parental strains were the same as in our previous study [25] and the framework of the protocol is identical for all the strains, as previously described, with the exception that some biochemical measurements differ among the different consomic strains, as described below.

Briefly, 15 weeks old male rats were equipped with telemetric radio-transmitters (TA11PAC40, Data Sciences International, St Paul, MN). After 3 weeks of recovery, BP was recorded in baseline conditions, during various salt loads (1 or 2% NaCl as drinking water) and after a washout period. 24-hours urine collections were also performed in baseline conditions, at the end of the salt load and at the end of the washout period. At the end of the study, 25 weeks-old animals fasted since 16h, were given a diazepam injection (5mg/kg, ip) and euthanized by decapitation.

Urine collection, blood and tissues sampling

A blood sample was drawn on heparin at the time of the sacrifice in fasted rats to measure creatinine, total cholesterol, triglycerides and glucose using Olympus automated tests (AU 2700 biochemistry analyzer, Olympus, Rungis, France), insulin (radioimmunoassay, DiaSorin, Antony, France) and plasma renin activity (PRA). For PRA-measurement, plasma contained additional ethylenediaminetetraacetate 20mM.

24-hours urines were used to measure creatinine, total proteins (pyrogallol red colorimetric method: AU 2700 biochemistry analyzer, Olympus, Rungis, France), sodium and potassium (flame photometer IL 943, Instrumentation Laboratory, Paris, France).

The left ventricle with septum was dissected out and weighed.

Measurement of plasma renin activity

Plasma renin activity was measured by trapping of generated angiotensin I by high affinity antibodies and subsequent radioimmunoassay [26].

Statistical analysis

Results are mean ± SEM. Statistical differences between strains were assessed using an independent t-test. LH rats were compared to BN rats and the consomic (LH-13^{BN} and BN-13^{LH}) were compared to their respective control (LH and BN rats).

Differences were considered statistically significant at $p<0.05$.

RESULTS

Body weight

Figure 1 shows, as previously described [25], that LH rats exhibit a much larger body weight than BN rats. Interestingly, LH-13^{BN} rats showed a significantly larger body weight than LH rats, while body weight was lower in BN-13^{LH} rats than in BN parents.

Cardiovascular parameters

Because differences in systolic, diastolic and mean BP (MBP) in the four strains are similar, only MBP values are presented here (Figure 2). Under baseline conditions, MBP of LH rats is approximately 40mmHg higher than that of BN rats (126 ± 0.9 mmHg vs 88 ± 1.6 mmHg, $p<0.01$). LH-13^{BN} rats exhibited a markedly lower baseline MPB than LH rats (113 ± 1.9 mmHg vs 126 ± 0.9 mmHg, $p<0.01$) while BN-13^{LH} rats showed no difference in MBP compared to BN rats (90 ± 1.6 mmHg vs 88 ± 1.6 mmHg). These MBP differences persisted throughout the study.

As shown by Figure 3, 2% NaCl in the drinking water resulted in a more marked MBP increase in LH rats than in BN. In LH-13^{BN}, MBP was less affected by NaCl 2% than in LH rats, although there was a 24 mmHg difference between LH and LH-13^{BN}. BN-13^{LH} did not differ from BN rats.

The relative (mg/100g of body weight) left ventricle (LV) weight was higher in LH than in BN rats (247 ± 1mg/100g vs 199 ± 4mg/100g, $p<0.01$). In LH-13^{BN}, the relative LV weight was decreased compared to LH rats (219 ± 3mg/100g vs 247 ± 1mg/100g, $p<0.01$) while, in BN-13^{LH} rats, there was a non-significant increase in relative LV weight compared to BN rats (207 ± 3mg/100g vs 199 ± 4mg/100g).

Renal function and plasma renin activity (Table 1)

The glomerular filtration rate (GFR), approximated by creatinine clearance, did not consistently differ among the four strains. However, urinary excretion of proteins was 6 to 11 fold greater in LH than in BN rats. BN-13^{LH} exhibited the same proteinuria as the BN while, in LH-13^{BN} rats, urinary excretion of proteins was 50% lower than that of LH parents.

Under baseline conditions, relative urinary excretion of Na^+ did not consistently differ among the four strains. However, at the end of the salt load, urinary excretion of Na^+ was significantly higher in LH than in BN rats. It was significantly lower in LH-13^{BN} than in LH while it was higher in BN-13^{LH} than in BN parents.

As shown by Table 1, before and after salt load, the Na/K ratio was lower in LH and LH-13^{BN} than in BN and BN-13^{LH}. At the end of the salt load, i.e. in 23 weeks old rats, these differences were less marked and the BN-13^{LH} exhibited the higher Na/K ratio.

LH rats showed a lower plasma renin activity than BN rats (Table 1). However, LH-13^{BN} and BN-13^{LH} rats exhibited the same level of plasma renin activity as their parental controls.

Metabolism (Table 2)

Plasma total cholesterol and triglycerides were higher in fasted LH than in BN rats. Plasma total cholesterol was markedly decreased in LH-13^{BN} rats compared to LH rats but remained higher than in BN-13^{LH} and BN rats. LH-13^{BN} also showed decreased plasma triglycerides compared to LH rats while BN-13^{LH} exhibited the same level of triglycerides as BN rats.

In fasted conditions, the four strains exhibited similar glycemia; however, the insulin- to-glucose ratio was significantly elevated in both LH and LH-13^{BN} compared to BN and BN-13^{LH}. No significant differences were found between the consomics and their respective parental strains.

DISCUSSION

We developed consomic rats, which differ from their parents by less than 5% of their genome (RNO13), because this approach provides a well-controlled model to dissect the genetic and physiological components of complex diseases such as BP and metabolic abnormalities due to a locus or loci on a single chromosome. Using this approach in RNO13 reciprocal consomics allowed us to confirm the effects of LH chromosome 13 on blood pressure regulation and also allowed us to identify novel effects of LH RNO13 on plasma lipids, protein excretion, and body weight.

While the most likely choice for consomics might have been LH and its commonly used normotensive control LN (Lyon Normotensive), the high genetic similarity between these two strains, simultaneously selected from the same colony of outbred Sprague Dawley, and the lack of markers available at the beginning of the project greatly reduced the ability to ensure substitution of the entire chromosome. Therefore, in order to substitute RNO13 in its entirety, we sought to use a donor strain that was more genetically diverse – BN. Our results from introducing the BN RNO13, were not completely reflective of the results from the previous linkage study involving the LH and LN rat strains. This could be due to the effects of a mixed genome background in the LH × LN cross or due to the genome differences between the LN and BN strains. Likely both scenarios are playing a role. However, the use of the BN as a chromosome donor offered some distinct advantages for genetic dissection of hypertension and phenotypes related to the metabolic syndrome in the LH rat. The LH and LN strains share over 90% genetic identity, as evidenced by the genotyping of >20,000 SNPs spanning the genome [27], while LH and BN share less than 5% genome identity. Therefore, the high genetic similarity between the LH and LN means many disease susceptible alleles would remain unidentified as they are actually shared between the strains. By introgressing the BN RNO13, we recapitulated the blood pressure phenotype on RNO13 that also mapped in the genetic cross between the LH and LN. Furthermore, we were able to identify novel loci where LH confers susceptibility to dyslipidemia and related features of the metabolic syndrome. In and of itself, the consomic strain has uncovered several interesting phenotypes that have genetic effects initiated by chromosome substitution. Indeed, this could have been our starting point, rather than first performing a large mapping cross followed by consomic dissection. Because we have the ability to generate overlapping congenics strains derived from the consomics, we now are able to dissect the phenotypes and to identify those genes, which is a distinct advantage of mapping using consomics compared to genetically unique F2 offspring that cannot be further studied after the initial mapping. Moreover, the genome of the BN strain has been sequenced [28], extensively characterized [29] and used as normotensive control strain for the development of consomic strains in other model of hypertension [30,31].

The integration of a BN RNO13 in the LH genetic background induced significant differences from the parental LH while the reciprocal consomic (the integration of a LH

RNO13 in the BN background) did not always show a phenotypic difference. It is likely that additional background susceptibility factors are required which are not found in BN rat, as has been seen in other studies [32] or that the BN rat is genetically resistant to hypertension and other characteristics of the metabolic syndrome. It is also likely the BN genome is highly resistant to high blood pressure and that the introduction of a single susceptibility factor from the LH is not sufficient to overcome homeostatic pressure keeping blood pressure regulated. This is not a new concept by any means, even when generating consomics and congenics from the same strains as used in mapping crosses. For example, in one study we recently published [33], introgression of all three major BP QTL alleles from the Genetically Hypertensive (GH) rat, independently or in a triple congenic, onto the background of the BN rat was not sufficient to significantly raise blood pressure. Conversely, consomics are very useful for identifying additional loci in the absence of genome background effects. For example, in an F2 male cohort from a mapping cross between the Dahl Salt Sensitive (SS) and the normotensive BN rats [34], a blood pressure QTL was not determined on RNO13, yet the SS-13^{BN} consomic shows a significant decrease in blood pressure and is being studied extensively for additional cardiovascular phenotypes [30,35,36,37]. Importantly, the goal of our work is to identify the factors that alter the phenotypes in the LH rat. Therefore, the fact that replacing the LH RNO13 with that of a highly resistant BN, resulted a clear and significant decrease in blood pressure, allows us to generate congenic lines with clear phenotypic differences and increases our likelihood of identifying the disease-causing alleles. Given we have the reciprocal consomics available (BN-13^{LH}), we could also pursue the mapping of BN modifier genes by an F2 intercross between BN-13^{LH} and LH.

Measures of plasma lipids showed LH rats have higher plasma concentrations of cholesterol and triglycerides than BN rats, similar to that of LN controls [15]. The introduction of a BN RNO13 in a LH genetic background reduced triglycerides and cholesterol, while the introduction of an LH RNO13 in a BN background did not affect these parameters (again we would hypothesize BN has strong compensatory mechanisms to regulate plasma lipids). This suggests that RNO13 is involved in the spontaneous hyperlipidemia shown by LH rats. The previous linkage study failed to reveal any QTL influencing plasma lipids on this chromosome [22]; the introduction of a different donor strain or the differences in the genome background can lead to the identification of new loci influencing a trait [23,38]. The substitution of the LH RNO13 markedly reduced plasma triglycerides. Interestingly, in our previous study [25], the replacement of LH RNO17 with that of the BN, also reduced plasma triglycerides to the level observed in BN parents suggesting a strong independent effect of RNO17 on hyperlipidemia or a common pathway with major components on both chromosomes. Taken together, these results allow us to hypothesize that the hypertriglyceridemia observed in LH rats is controlled by loci in at least two chromosomes (RNO17 and RNO13). It remains to be seen if there is epistatic relationships between these loci or if they fall within independent regulatory pathways. The generation of double consomic (LH-17^{BN}.13^{BN}) will help address potential interactions.

Interestingly, LH-13^{BN} rats have a significantly increased body weight compared to LH, and BN-13^{LH} a decreased body weight compared to BN. Such a result was unexpected. First, as the LH rats are significantly heavier than BN, we would have expected the substitution of an LH RNO13 with that of a BN would *reduce* body weight and, conversely, the substitution of a BN chromosome with that of LH would result in higher body weight. Furthermore, our genetic linkage studies between LH and LN rats [21,22] failed to reveal significant body weight QTL on RNO13. Our results suggest that a gene(s) on LH RNO13 actually contributes to reduced weight, but that genes on other chromosomes (e.g. RNO1 and RNO17) play a stronger role and determine the overweight phenotype seen in the LH rat [22,25]. In further studies, it will be important to investigate if the modification of body

weight is the consequence of a change in height or in adiposity and to evaluate the repartition of this adiposity. However, what is intriguing is that the consomic strains have allowed us to clearly partition body weight from dyslipidemia as both reciprocal consomics show marked opposite effects of chromosome substitution on body weight and plasma lipid levels.

Under baseline diet conditions, mean blood pressure is decreased in LH-13^{BN} compared to LH rats. This difference is more marked than previously observed in LH-17^{BN} consomic rats [25]. Thus the substitution of RNO13 independently influences blood pressure in LH rats. However, the converse is not true: the introduction of a LH RNO13 in a BN background did not significantly increased BP. During salt load, BP remained stable in BN while it increased gradually in BN-13^{LH}, LH-13^{BN} and LH rats. This demonstrates that RNO13 is partially implicated in the salt sensitivity observed in LH rats. Similar data were reported in Dahl salt sensitive rats in which the introduction of a BN RNO13 conferred protection from salt induced hypertension [30]. Recently the generation of overlapping congenics from these consomic rats demonstrated that the RNO13 contain 4 regions that interact epistatically. Among these, two of them were sex specific [36].

Similarly, the urinary Na/K ratio, which is inversely related to mineralocorticoids activity, was lower in LH and LH-13^{BN} compared to BN and BN-13^{LH}. This is in accordance with the increase in mineralocorticoids found in LH rats [39]. However, the lack of significant difference due to the substitution of RNO13 suggests it is independent of RNO13.

In LH-13^{BN}, total urinary excretion of proteins is reduced compared to LH parents. However, because the reduction in protein excretion could also be the consequence of the decrease of the deleterious effects of hypertension on the kidneys, we can not definitively conclude that this reduction is directly influenced by specific RNO13 genes. However, generation of congenic substrains should enable us to examine independent and pleiotropic gene effects.

Finally, this study allowed us to confirm that LH rats are a model of low renin hypertension [40]. Because LH-13^{BN} and BN-13^{LH} rats exhibit the same level of plasma renin activity as their controls (LH and BN rats, respectively) and LH and BN rats differ by a polymorphism of the intron A of the renin gene [41], it is unlikely that the level of plasma renin activity is related to this polymorphism, and may in fact be regulated by loci on another chromosome, i.e. in trans. We can also conclude that, in our model, an important part of hypertension is independent of plasma renin activity level.

Taken together these results allowed us to dissect characteristics of LH rats related to hypertension and metabolic syndrome. It is interesting to observe that several parameters such as BP, protein excretion and plasma lipids evolved similarly and independently of plasma renin activity. Although this study does not allow us to entirely exclude renin as a candidate gene for spontaneous hypertension in the LH rat, it demonstrates that, if the renin gene is a determinant of essential hypertension in our model, this occurs independently of plasma renin activity.

According to the current data in the Rat Genome Database, which is responsible for curating all rat genes, there are 826 genes on rat RNO13.To identify other potential candidate gene, we queried PubMed for genes on RNO13 annotated with the terms ‘hypertension OR blood pressure OR lipids OR metabolic syndrome’. Thirteen genes in addition to Renin were identified: Adora1 (adenosine A1 receptor); Ptgs2 (prostaglandin-endoperoxide synthase 2; aka Cox2); RGD1307782 (similar to Williams-Beuren syndrome critical region protein 27); Atp1a2 (ATPase, Na+/K+ transporting, alpha 2 polypeptide); Soat1 (sterol O-acyltransferase 1); Avpr1b (arginine vasopressin receptor 1B); Hsd11b1 (hydroxysteroid 11-

beta dehydrogenase 1); LOC679692 (similar to lysophosphatidylglycerol acyltransferase 1); Selp (selectin, platelet); Myoc (myocilin); Rgs2 (regulator of G-protein signaling 2); Rgs5 (regulator of G-protein signaling 5); Prdx6 (peroxiredoxin 6). Until we have generated and characterized congenic strains derived from the consomic strains, we cannot formally include or exclude any of these, but they are candidates for follow-up in congenic and gene expression studies.

In conclusion, this work shows that the consomic strains approach is a useful step in the dissection of a highly complex disease like hypertension and components of the metabolic syndrome. However, one of the limitations of this kind of study is that loci mapped as a single QTL are more often composed of multiple QTLs with small and/or opposing effects on the trait of interest. Therefore the observed phenotype in the consomic strain is the sum of all allelic variation between the parental strains. Since we demonstrated here that RNO13 of LH rats has a significant influence on hypertension we can use these consomic animals to develop overlapping congenic strains [42] for further dissection of the factors on RNO13. The same strategy permitted Moreno et al. to identify four regions within RNO13 influencing blood pressure in SS rat with sexual dimorphisms [36]. By this approach, the study of males and females in overlapping congenics derived from our consomic lines may allow us to further understand the complex actions and interactions in regulating BP and metabolic abnormalities shown by LH rats. Interestingly, there is a very close phylogenetic relationship between the SS and the LH rats [27]. Given the fact that the same BN donor strain (BN/NHsdMcwi) was used in this study as that of the SS-13^{BN} strain [36], future genotype-phenotype correlations could utilize these genetic similarities in assessing candidate genes or modifier gene for common traits measured in each consomic or parental lines.

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Abbreviations

BP	Blood Pressure
BN	Brown Norway
GFR	Glomerular Filtration Rate
GH	Genetically Hypertensive
LH	Lyon Hypertensive
LN	Lyon Normotensive
LV	Left Ventricle
MBP	Mean Blood Pressure
PRA	Plasma Renin Activity
QTL	Quantitative Trait Locus
RNO13	Rat Chromosome 13
SHR	Spontaneous Hypertensive Rat
SS	Dahl Salt Sensitive

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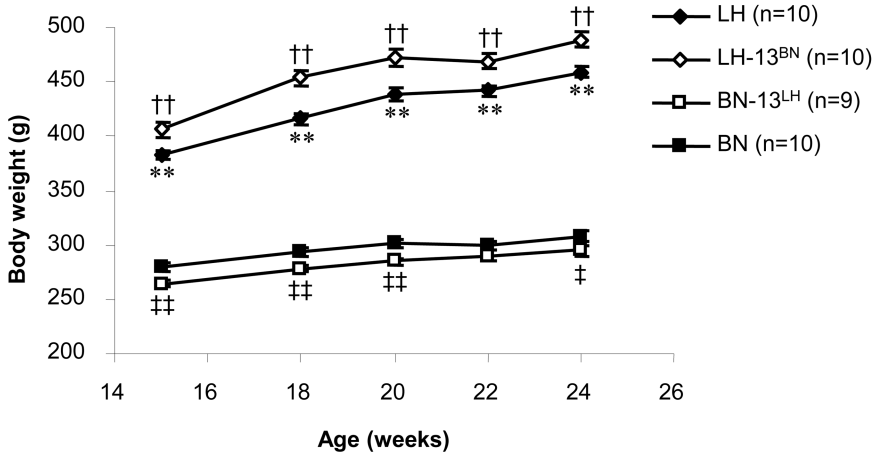

Figure 1.

Time course evolution of body weight in LH (n=10), LH-13^{BN} (n=10), BN-13^{LH} (n=9) and BN rats (n=10). ** $p<0.01$ LH *versus* BN; †† $p<0.01$ LH-13^{BN} *versus* LH; ‡ $p<0.05$, ‡‡ $p<0.01$ BN-13^{LH} *versus* BN.

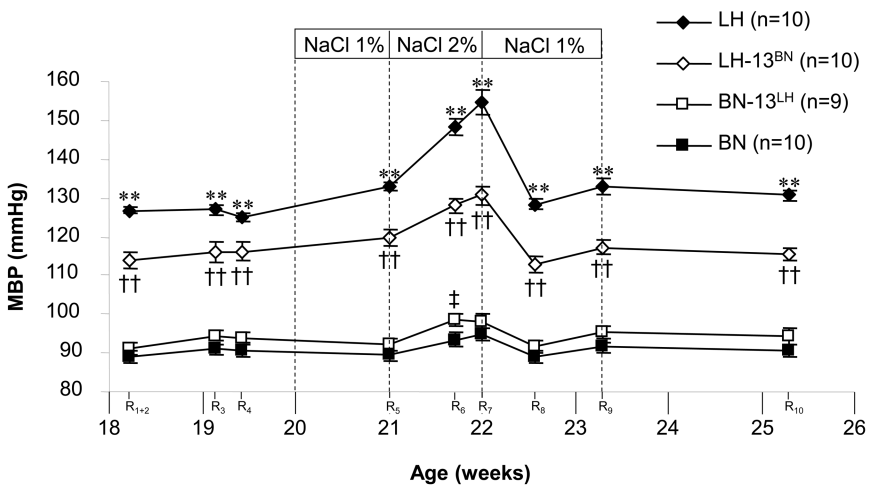

Figure 2.

Time course of mean blood pressure (MBP) recorded by radio telemetry in LH rats (n=10), LH-13^{BN} (n=10), BN-13^{LH} (n=9) and BN rats (n=10). The telemetric radio-transmitters were implanted at 15 weeks of age. The blood pressure recording periods were indicated by the abbreviation R_X (R_1 for the first recording to R_{10}). Each MBP value corresponds to the average of a 22-h beat-to-beat MBP recording. The values of the first 2 recordings (R_1, R_2) were averaged to obtain the baseline value (R_{1+2}). The top of the graph shows the nature and the chronology of the different salt load (1% and 2% NaCl in drinking water). ** $p<0.01$ LH *versus* BN; †† $p<0.01$ LH-13^{BN} *versus* LH; ‡ $p<0.05$ BN-13^{LH} *versus* BN.

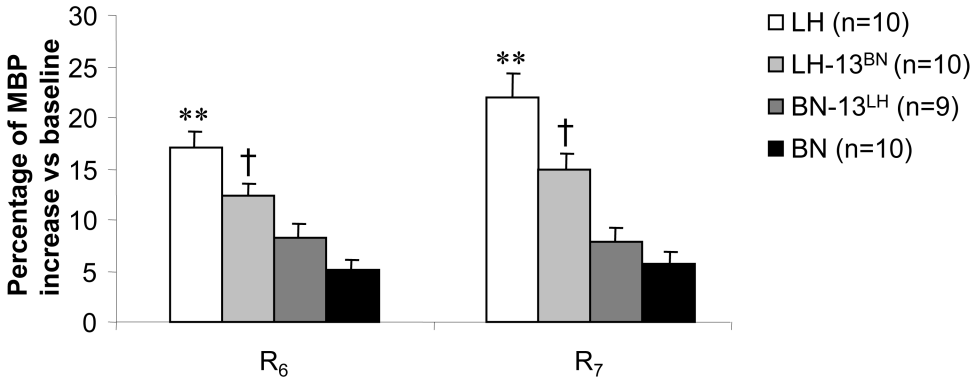

Figure 3.

Percentage of mean blood pressure increase versus baseline value (R_{1+2}) during high salt load (2% NaCl as drinking water). R_6 and R_7 correspond respectively to 5 and 7 days under high salt load. ** $p<0.01$ LH *versus* BN; † $p<0.05$ LH-13^{BN} *versus* LH.

Table 1

Renal function and plasma renin activity in LH, LH-13^{BN}, BN-13^{LH} and BN rats.

	BN n=10	LH n=10	LH-13^{BN} n=10	BN-13^{LH} n=9
GFR (mL/min/100g)				
19 W	0.46±0.03	0.45±0.01	0.49±0.01 ††	0.49±0.02
23 W	0.44±0.03	0.46±0.01	0.47±0.01	0.55±0.01 ##
25 W	0.44±0.02	0.46±0.01	0.45±0.02	0.49±0.02
U Prot (mg/24h/100g)				
19 W	4.98±0.29	30.59±2.64 **	15.21±1.44 ††	5.71 ±0.81
23 W	5.29±0.23	57.68±4.34 **	21.88±2.50 ††	5.40±0.30
25 W	5.27±0.21	55.26±3.51 **	22.47±2.38 ††	5.20±0.73
U Na (mmol/L/100g)				
19 W	0.49±0.04	0.49±0.04	0.42±0.02	0.65±0.02 ##
23 W	1.32±0.09	2.76±0.24 **	2.00±0.08 ††	1.95±0.09 ##
25 W	0.44±0.03	0.45±0.02	0.43±0.02	0.61 ±0.03 ##
Na/K Urinary				
19 W	0.21 ±0.02	0.12±0.01 **	0.11±0.01	0.21 ±0.01
23 W	0.56±0.02	0.52±0.03	0.52±0.02	0.64±0.02 ##
25 W	0.20±0.01	0.10±0.01 **	0.12±0.01	0.21 ±0.01
PRA (ng/mL/h)				
25 W	24.60±2.51	11.62±2.24 **	12.19±1.15	24.33±2.53

Renal function in LH (n=10), LH-13^{BN} (n=10), BN-13^{LH} (n=9) and BN rats (n=10) at the age of 19 weeks (baseline diet condition), 23 weeks (1 % NaCl as drinking water) and 25 weeks (baseline diet condition).

GFR, Glomerular Filtration Rate (estimated by creatinine clearance); U Prot, Proteinuria; U Na, Relative excretion of sodium; PRA, Plama Renin Activity

** $p < 0.01$ LH *versus* BN

†† $p < 0.01$ LH-13^{BN} *versus* LH

$p < 0.01$ BN-13^{LH} *versus* BN

Table 2

Plasma characteristics in fasted 25 week-old LH, LH-13^{BN}, BN-13^{LH} and BN rats.

	BN n=10	LH n=10	LH-13BN n=10	BN-13^{LH} n=9
Cholesterol (mmol/L)	1.59±0.05 (9)	3.44±0.21 **	2.07±0.07 ††	1.58±0.03
Triglycerides (mmol/L)	0.57±0.02	0.99±0.08 **	0.71 ±0.05 ††	0.58±0.02
Glucose (mmol/L)	7.39±0.16	7.81 ±0.22	7.67±0.18	7.66±0.41
Insulin (mUI/L)	20.37±0.91	29.17±1.42 **	30.84±1.62	23.41 ±1.88
Insulin/glucose	2.76±0.13	3.80±0.25 **	4.02±0.23	3.06±0.17

Numbers in brackets indicate the number of rats if different.

** $p < 0.01$ LH *versus* BN

†† $p < 0.01$ LH-13^{BN} *versus* LH

‡ $p < 0.05$ BN-13^{LH} *versus* BN