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EDITORIAL

Recombinant Inbred Strains in Hypertension Research

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Summary

It was recognized that recombinant inbred strains are a very powerful system for the study of the genetics of hypertension, linkage analysis and gene mapping. Such set of recombinant inbred strains has been developed in the cooperation of Prof V. Křen and Dr. M. Pravenec in Prague. These recombinant inbred strains were used to search for the genes of spontaneous hypertension and to test the phenotypic differences. It was found that 1) the major histocompatibility compex of the rats showed a significant association with blood pressure, 2) the restriction fragment lenght polymorphism in kallikrein gene family as well as renin gene cosegregated with blood pressure, 3) Na^+ leak in red blood cells cosegregated with blood pressure, 4) the relative heart and kidney weights are not closely related to mean arterial pressure and 5) the platelet aggregation and blood pressure are independent traits. The results indicate the usefulness of recombinant inbred strains in the analysis of the relationship between phenotype and genotype.

It is widely accepted that the quantitative trait, blood pressure, has a polygenic genetic component (Hansen 1972, Yen *et al.* 1974, Tanase *et al.* 1970, 1972, Williams *et al.* 1988). The understanding of human essential hypertension requires a detailed analysis and description of the genetic loci involved but this is difficult to study in an outbred human population. A using of the inbred animal models is much more useful but it has also some limitations.

The study of the genetics of hypertension is not easy and several different experimental approaches have been developed to address this issue (Fig. 1). The simplest way is to compare two inbred strains, one normotensive and one hypertensive. The spontaneously hypertensive rat (SHR) is the most widely studied genetic model of hypertension (Okamoto and Aoki 1963). However, the question which strain constitutes the adequate experimental control strain for comparison with SHR, has been recently intensively discussed (Kurtz and Morris 1987, Rapp 1987, Kurtz *et al.* 1989, St.Lezin *et al.* 1992, Lindpainter *et al.* 1992). Though many other genetic models of hypertension were developed (for review see Yamori 1982), the problem still exists that the multiple genetic differences between hypertensive strain and their normotensive controls have usually no relation with hypertension.

Fig. 1
The main approches for the study of the genetics of hypertension

* Genetic models
* Genetically segregated populations
  - F_2 hybrids
  - Recombinant inbred strains
  - Recombinant congenic strains
* Transgenic animals

The better way than a simple comparison of two inbred strains is to cross-breed normotensive and hypertensive animals (Rapp 1983). All animals in the F_1 generation will be heterozygous, each carrying one hypertensive and one normotensive allele. In the F_2 generation, there will be a segregation into homozygous hypertensive, homozygous normotensive and heterozygous (hypertensive/normotensive) genotypes. In a large F_2 population one can correlate individual blood pressure values with other measured parameter to look if

there is any relation among the traits of interest. Various mathematical methods of analyzing quantitative traits controlled by multigenic systems in segregating populations were developed (Falconer 1963, Roderick and Schlager 1966). The main disadvantage of this approach is that a quantitative phenotype cannot be established reliably in a single individual and thus the analysis of the relationship between phenotype and genotype is very difficult. This difficulty was recognized by Bailey (1965, 1971, 1981) who produced a series of recombinant inbred strains to analyze various quantitative traits.

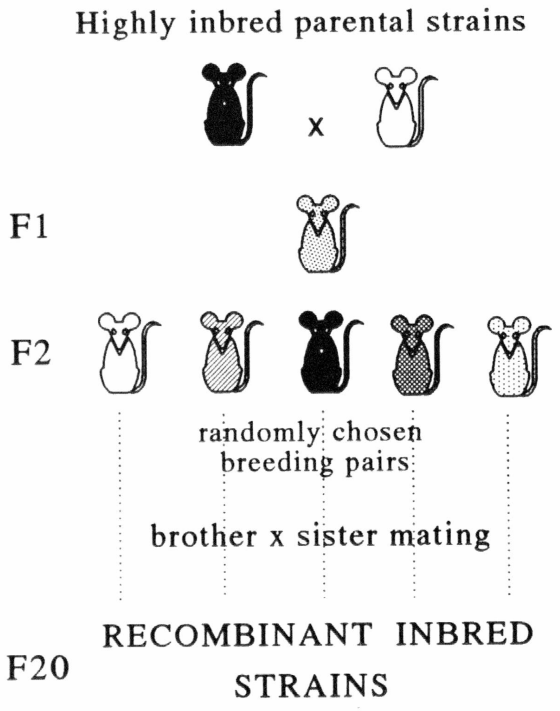

Fig. 2
The scheme of breeding methods necessary for the development of recombinant inbred strains.

Rationale for the use of recombinant inbred strains

Recombinant inbred (RI) strains are produced by brother-sister mating between members of F_2 generation resulting from a cross of two inbred parental strains (Fig. 2). Because RI strains are inbred they are considered to be homozygous at each locus. Thus strain distribution pattern for polymorphic allele can be established by determining for each RI strain, which parental alleles are inherited at particular loci. Since the RI strains are developed in the absence of a genetic selection, the particular homozygous allele at each locus is determined only by the random segregation and crossover events. The fact that each recombinant genotype of particular RI strain is replicable in an indefinite number of individuals within this strain is an extremely powerful advantage over the classical F_2 approach. This property allows linkage analysis of traits that could not be made in a single individual. Several traits that cannot be measurable in the same individual could be also analyzed by this method.

The unique set of RI strains of rats was developed by Prof. V. Křen (Dept. of Biology, Faculty of Medicine, Charles University) and Dr. M. Pravenec (Institute of Physiology, Czech Academy of Sciences) in Prague (Czech Republic). They used randomly chosen members of F_2 generation resulting from the cross between spontaneously hypertensive rats and Brown Norway.lx (BN) normotensive ones (Pravenec *et al.* 1989). In the first study 31 RI strains at F_{16} to F_{17} generations were used for blood pressure determination. Systolic, diastolic and mean arterial pressures were measured by a direct punction of the carotid artery under light ether anaesthesia, which is a standard method used in our laboratory. Blood pressure values of RI strains were continuously distributed between both progenitor strains with slight tendency to be closer to normotensive values (Fig. 3). We have also found a positive correlation ($r = 0.731$, $p<0.001$) between average values of mean arterial pressure in particular inbred strains examined in F_{16} and F_{23} generations suggesting a considerable stability of blood pressure (Kuneš *et al.* 1990). Because RI strains could not be separated according to their blood pressure values into distinct phenotypic groups, we suggested that three major genes and multiple minor genes might be involved in the determination of spontaneous hypertension (Pravenec *et al.* 1989).

The use of RI strains to search for the genes responsible for hypertension

Until now RI strains were typed for many morphological, biochemical and immunological markers to search for the genes responsible for high blood pressure. We have found that RT1 complex (major histocompatibility complex of the rat) showed a significant association with blood pressure (Pravenec *et al.* 1989). The mean arterial pressure of RI strains sharing SHR allele was significantly higher in comparison with RI strains sharing BN allele (136 ± 3 vs 124 ± 2 mm Hg, $p<0.001$). One of the genes of RT1 complex responsible for this blood pressure difference could be HSP 70 gene (Hamet *et al.* 1992). Restriction fragment length polymorphism for this gene is in a good agreement with a sharing of particular allele of individual RI strains (Fig. 4).

Another way how to test the role of any gene(s) in the determination of a quantitative trait is to develop recombinant congenic strains which are produced by limited backcrossing between two strains and subsequent brother-sister mating (Démant and Hart 1986). In this way a small fraction of the genome of one strain is transferred on the genetic background of the second strain.

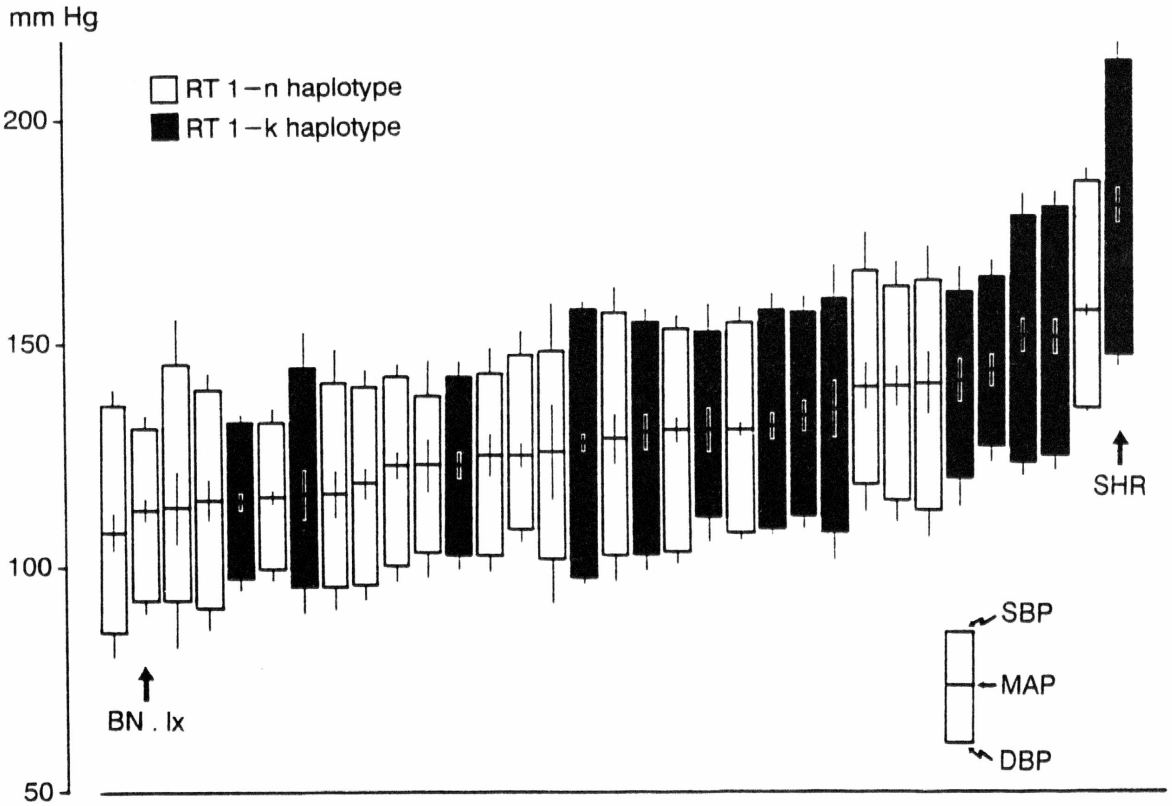

Fig. 3
Systolic (SBP), mean arterial (MAP) and diastolic (DBP) blood pressures in adult males of recombinant inbred strains. Blood pressure values of both progenitors are indicated by arrows.

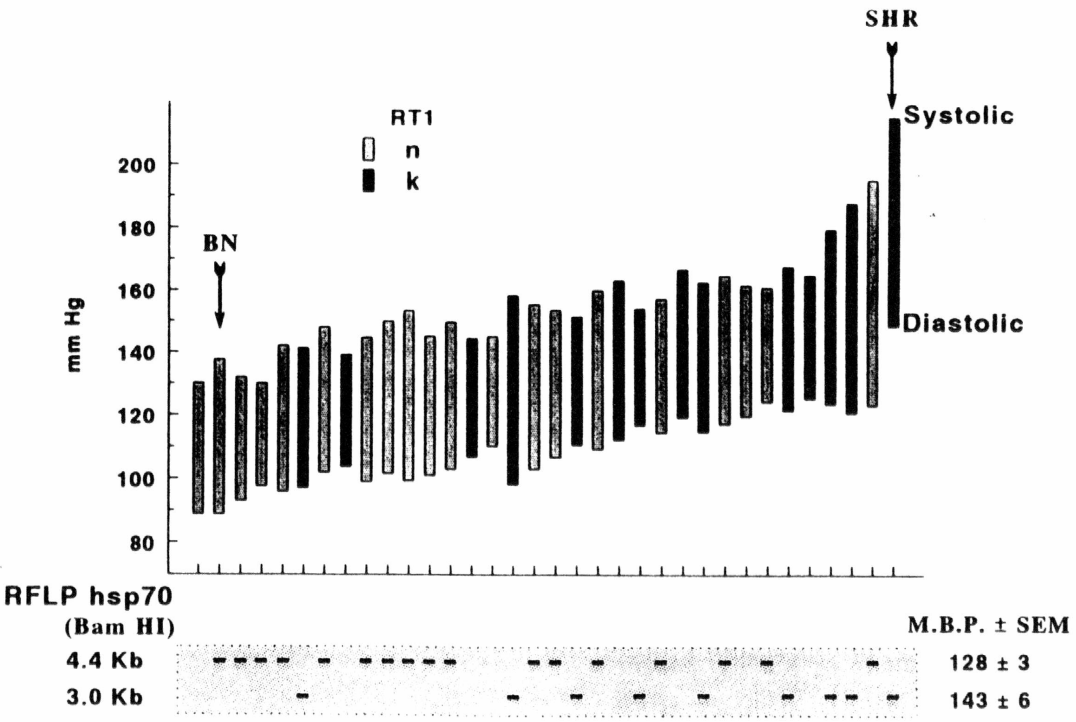

Fig. 4
Blood pressure and restriction fragment length polymorphism of hsp 70 in recombinant inbred strains. (with permission from Hamet *et al.* 1992)

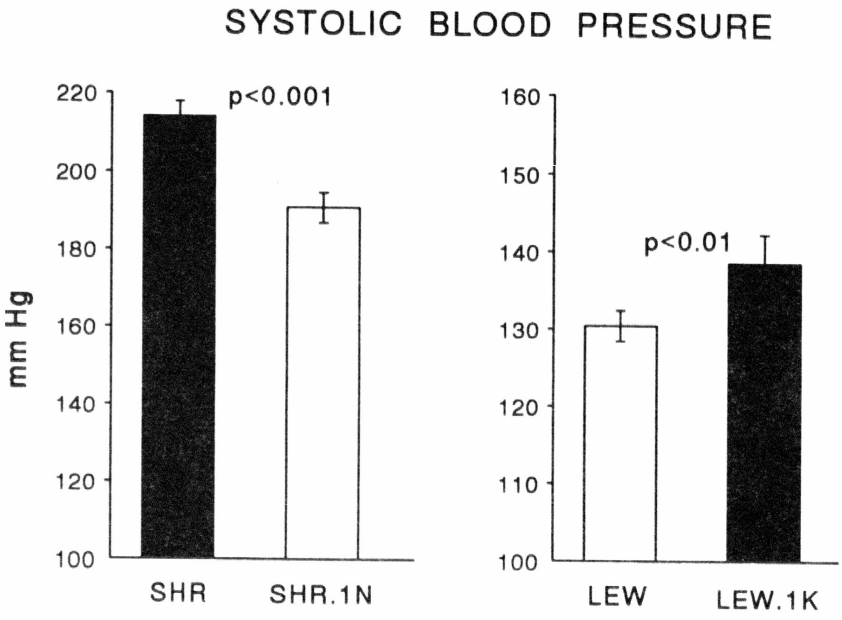

Fig. 5
Systolic blood pressure of SHR and their SHR.1N congenic strain as well as of normotensive LEW strain and its LEW.1K congenic strain. Data are mean ± SEM.

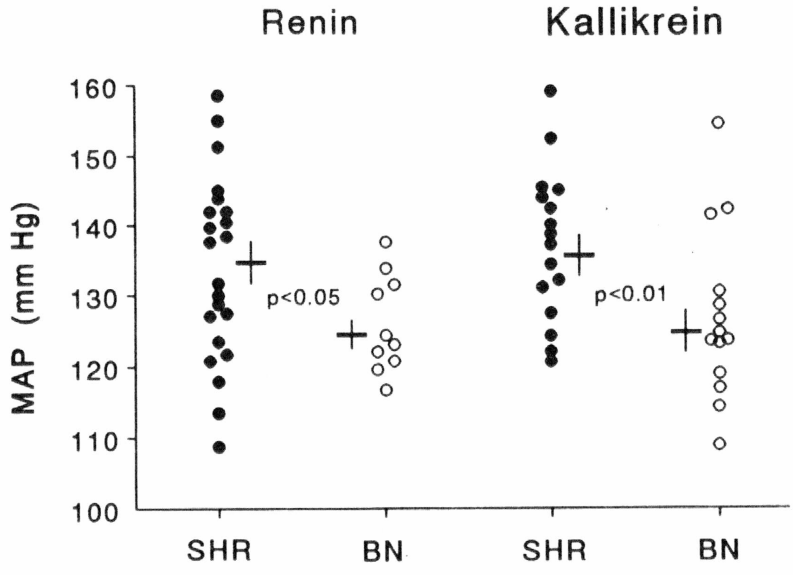

Fig. 6
Scatter diagram of mean arterial pressure (MAP) for RI strains inherited either BN allele or SHR allele for renin and kallikrein gene, respectively. Horizontal lines represent the average values with SEM.

To check how strong is the influence of the genes within RT1 complex on blood pressure we studied SHR.1N congenic strain in which $RT1^n$ haplotype from BN strain was transferred on the genetic background of SHR. We also used a LEW.1K congenic strain with the genetic background of normotensive Lewis (LEW) strain and $RT1^k$ haplotype from SHR. It is evident (Fig. 5) that the genes within $RT1^n$ haplotype were able to decrease blood pressure of SHR and on the contrary the genes within $RT1^k$ haplotype increased blood pressure of normotensive LEW strain.

The set of RI strains was also used for the linkage study of blood pressure increase with a sequence alterations in the kallikrein gene family and in the renin gene (Pravenec *et al.* 1991 a,b). The decreased urinary kallikrein excretion has been described in at least three different rat models of genetic hypertension (Carretero *et al.* 1976, Carretero *et al.* 1978, Ader *et al.* 1987). Several studies in different animal models of hypertension have provided evidence that the occurrence of structural alterations in the renin gene (Samani *et al.* 1989, 1990, Dene *et al.* 1989) might have the capacity to affect blood pressure (Kurtz *et al.* 1990, Rapp *et al.* 1989). We have found that the restriction fragment length polymorphism (RFLP) in kallikrein gene family and renin gene cosegregated with blood pressure in our set of RI strains (Pravenec *et al.* 1991 a,b). Fig. 6 shows that mean arterial pressure of RI strains is stratified according to kallikrein genotype or renin genotype. Blood pressure of RI strains that inherited RFLP marking SHR kallikrein gene family or SHR renin gene was significantly higher when compared to blood pressure of RI strains inherited respective BN alleles.

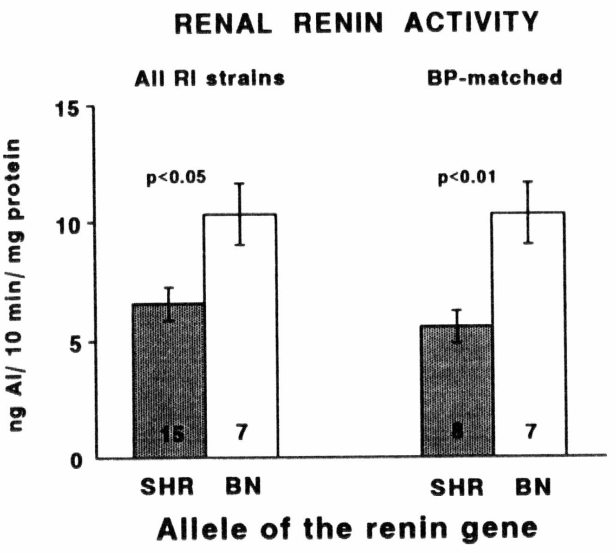

Fig. 7
Renal renin activity for RI strains inherited either BN allele or SHR allele for renin gene.

Recently, we have demonstrated (Pohlová *et al.* 1993) that the major structural alterations in the renin gene described for SHR strain (Samani *et al.* 1989, Kurtz *et al.* 1990, Lindpainter *et al.* 1990, Pravenec *et al.* 1991a) are accompanied by a reduction in renal renin activity in RI strains inheriting the SHR allele of the renin gene (Fig. 7). This could not be a secondary influence of high blood pressure because the same was seen even in blood-pressure matched RI strains. The mechanisms by which structural abnormalities of the renin gene might influence the renin level in the kidney remain to be elucidated.

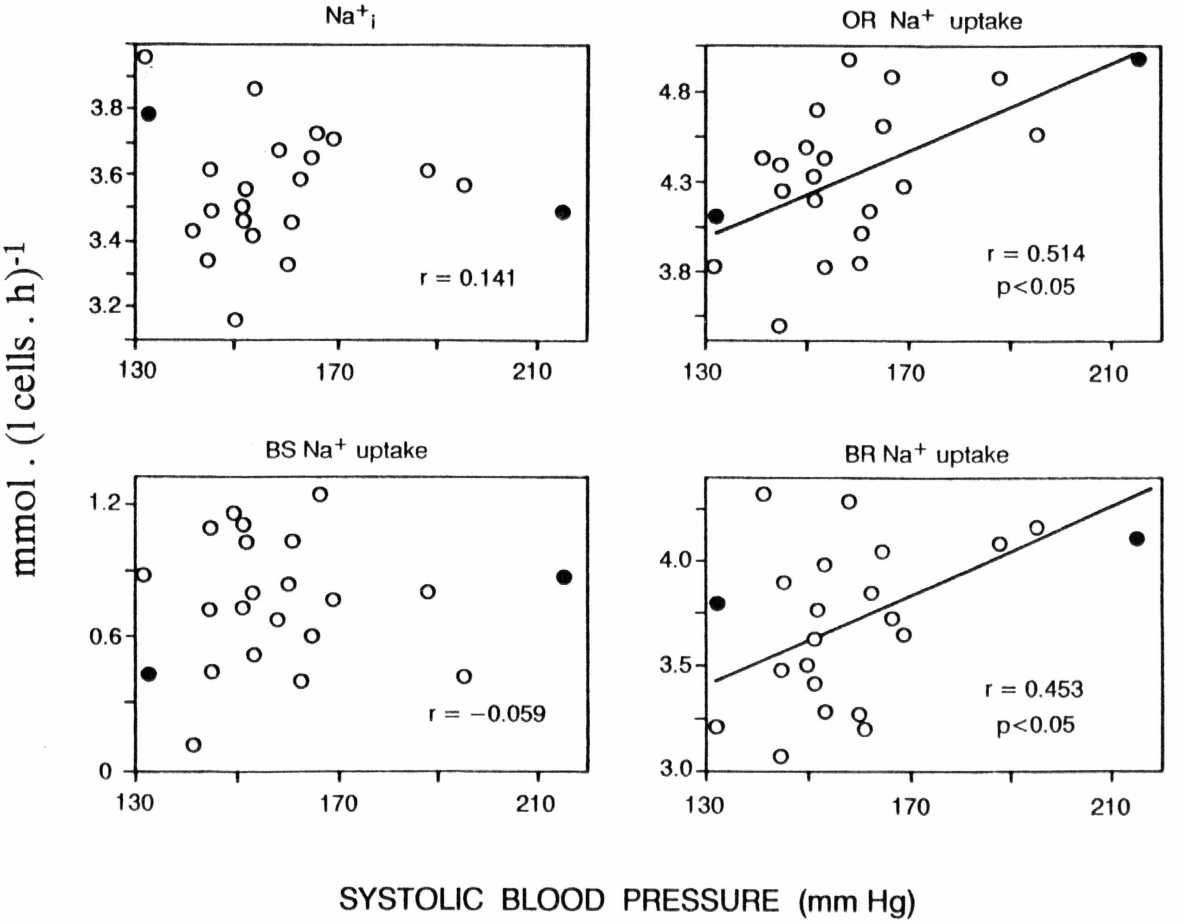

Fig. 8
Correlations of red blood cell Na^+ content (Na^+_i), ouabain-resistant (OR) Na^+ uptake as well as bumetanide-sensitive (BS) and bumetanide-resistant (BR) Na^+ uptake with systolic blood pressure. open circles – RI strains (five rats of each), closed circles – progenitor strains.

RI strains in search for the phenotypic differences

Ion transport abnormalities have been proposed to play an important role in the pathogenesis of human as well as experimental hypertension (Postnov and Orlov 1985, Aviv and Lasker 1990, Zicha *et al.* 1992). We used RI strains to investigate whether the increased passive membrane permeability for monovalent cations that is repeatedly described in rats with genetic hypertension (Orlov *et al.* 1991) is indeed related to high blood pressure. We have found that ouabain-resistant Na^+ net uptake as well as furosemide- and bumetanide-resistant Na^+ inward leaks in red blood cells cosegregated with systolic (Fig. 8) and pulse pressures but not with diastolic pressure of RI strains. There were no correlations of blood pressure with red cell Na^+ content or furosemide- and bumetanide-sensitive Na^+ net uptakes. Cosegregation of Na^+ leak with blood pressure supports its importance in the pathogenesis of hypertension under the assumption that increased Na^+ influx and cell Na^+ turnover also occur in some tissues directly involved in the cardiovascular regulation of genetically hypertensive animals (Bin Talib *et al.* 1992).

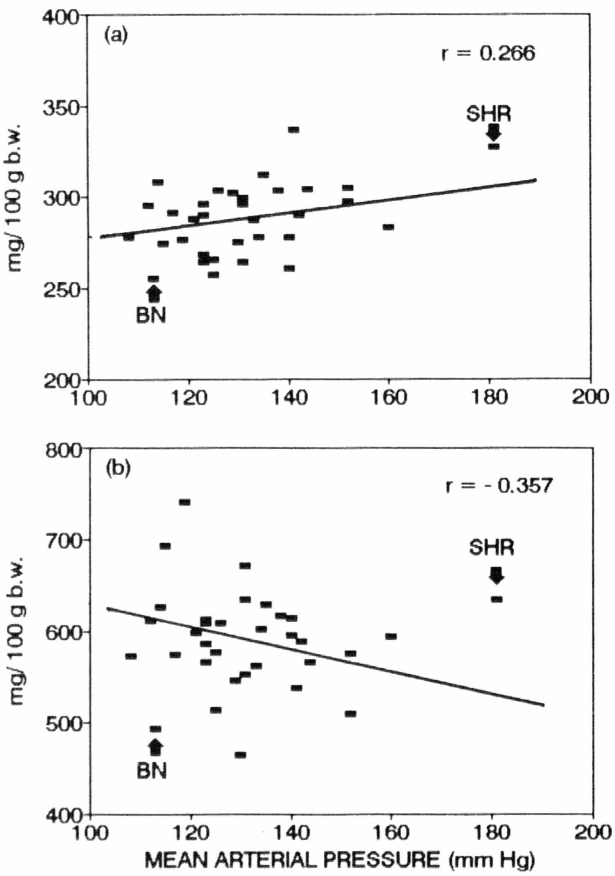

Fig. 9
The relationship of mean arterial pressure and relative heart (a) and kidney (b) weights in RI strains. Arrows indicated values of both progenitors.

As mentioned above multiple differences found between hypertensive and normotensive animals have usually nothing to do with hypertension. Cardiac and renal enlargements have been intensively investigated in hypertensive humans as well as in animals with genetic or induced hypertension. Recently, Pang *et al.* (1986) disclosed cardiac and renal hyperplasia in newborns of three different models of genetic hypertension but not in the offspring of parents with renal or DOCA-salt hypertension. On the other hand, we have found even lower relative heart weight in newborns of Prague hypertensive rats (Dobešová *et al.* 1990) although the cardiac hypertrophy was seen in adult rats. This suggests the dissociation of genes for the determination of organ weight from those responsible for the determination of blood pressure. Moreover, Tanase *et al.* (1982) found that heart weight is a highly heritable trait and that the effect of genetic factors on cardiac weight is larger than the effect of blood pressure. We have solved the question whether cardiac and renal enlargements are primary or secondary events in hypertension by using of our set of RI strains (Kuneš *et al.* 1990). The results shown that relative heart and kidney weights were not closely related to mean arterial pressure (Fig. 9) in RI strains and they were clustered closer to normotensive values. It was evident that several strains with a low blood pressure had nearly the same relative heart weight and even greater relative kidney weight than SHR progenitor, suggesting that organ weight in these strains must be determined genetically. The analysis of genetic determination of blood pressure and organ weights revealed that relative kidney weight shoved a high degree of genetic determination while the genetic determination of relative heart weight and mean arterial pressure was lower (Kuneš *et al.* 1990). The results of our study supported the earlier report of Tanase *et al.* (1982) on the influence of primary genetic factors on organ weights.

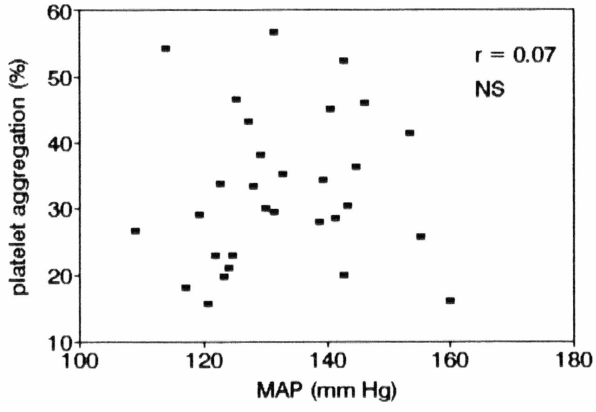

Fig. 10
Linear regression analysis of mean arterial pressure (MAP) and percentage aggregation in RI strains. Each point represents mean value for at least six individual rats from each RI strain.

The relationship between blood pressure and platelets aggregation was intensively studied in human essential hypertension and animal models of genetic hypertension. Although, contradictory results were obtained in human and animal studies (hyper-aggregability vs hypo-aggregability), the authors believe that the platelets may serve as an easily accessible model system of contractile cell function. It was also tested by using of RI strains if the alterations in platelet aggregation are related to hypertension. The typical platelet hypo-aggregability was found in SHR when compared with Brown Norway strain (Pravenec *et al.* 1992). Nevertheless, linear regression analysis of mean arterial pressure and aggregation in RI strains revealed that the platelet aggregation and blood pressure are independent traits (Fig. 10). These results indicated the usefulness of RI strains in the analysis of the relationship between phenotype and genotype. In the case of platelet aggregation is evident that platelet hypo-aggregability and spontaneous hypertension in SHR were linked together by chance due to drift during selective inbreeding.

Conclusion

It is evident that the set of recombinant inbred strains is a very powerful system for the study of genetics of hypertension, linkage analysis and gene mapping. It could be emphasize that in essential hypertension research each positive correlation between two traits, often found in population-based studies, should be reaffirmed in genetically better-defined systems, e.g. by pedigree analysis.

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