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Two point mutations within the adducin genes are involved in blood pressure variation

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ABSTRACT **The Milan hypertensive strain of rats (MHS) develops a genetic form of renal hypertension that, when compared to its normotensive control (MNS), shows renal dysfunction similar to that of a subset of human patients with primary hypertension. MHS and MNS were shown to be homozygous by multilocus minisatellite analysis and monolocus microsatellite markers. We show here that one point mutation in each of two genes coding for the membrane skeleton protein adducin is associated with blood pressure in the Milan strain of rats. Adducin is a heterodimer formed by α and β subunits that promotes the assembly of actin with spectrin. MHS and MNS differ, respectively, by the amino acids Y and F at position 316 of the α subunit. In the β-adducin locus, MHS is always homozygous for R at position 529 while in MNS either R or Q occurs in that position. The R/Q heterozygotes showed lower blood pressure than any of the homozygotes. *In vitro* phosphorylation studies suggest that both of these amino acid substitutions occur within protein kinase recognition sites. Analysis of an F_2 generation demonstrated that Y alleles segregated with a significant increment in blood pressure. This effect is modulated by the presence of the R allele of the β subunit. Taken together, these findings strongly support a role for adducin polymorphisms in causing variation of blood pressure in the Milan strain of rats.**

Primary or essential hypertension is a heterogeneous disease affecting 15–20% of the adult population (1). Different genetic mechanisms causing renal, endocrine, humoral, or nervous dysfunction have been suggested (2). Different rat models of genetic hypertension have been developed, and for three of them a genetic association between a DNA polymorphism and hypertension has been suggested (3–8). The Milan hypertensive strain of rats (MHS) was developed by selection for hereditary hypertension in divergence to its normotensive control (MNS), which was selected for low blood pressure. At present, 85 generations of inbreeding have been reached (9, 10). Compared to MNS, MHS shows a greater pressor effect of the kidney after transplantation (9, 11), a faster glomerular filtration rate and tubular reabsorption (9, 12), a lower kidney weight (9) and plasma renin activity (13), and a lower volume of erythrocytes (14) and tubular cells (15), both of which show faster Na transport across their plasma membrane (14, 16, 17). Erythrocyte functional differences are genetically determined within the stem cells and are genetically associated with hypertension in F_2 hybrids (18). Thus, a generalized and genetically determined cellular defect involving faster Na transport across the renal cell membranes (19) was considered a probable cause of hypertension in MHS. Some of the erythrocyte and kidney dysfunctions seen in these rats have also been found in a subset (≈25%) of human patients with primary hypertension (10, 20–22). In the rat model, the difference in membrane ion transport disappeared after elimination of the membrane skeleton, which indicated the involvement of some of its components (23, 24). Cross-immunizations between MHS and MNS raised an antibody against a membrane skeleton protein subsequently identified as adducin (25). As this was the only cytoskeletal difference found that could be associated with membrane ion transport differences, adducin was considered a candidate for genetic studies in hypertension.

Adducin is an $\alpha\beta$ heterodimer with subunits of M_r 103,000 (α) and 97,000 (β). It promotes the organization of a spectrin–actin lattice, a function regulated by phosphorylation and Ca–calmodulin interactions (26–28). Furthermore, α- and β-adducin have similarities with the MARCKS group of proteins, which are involved in cellular signal transduction mechanisms (29). We characterized the rat full-length β- and α-adducin cDNAs (ref. 30; G.T., G.C., G.B., and F.E.B., unpublished data) and localized the genes on chromosomes 4 and 14, respectively (G.C., G.T., G.B. and F.E.B., unpublished data). Corresponding human α- and β-adducin cDNAs were isolated from an erythroblastoid cell line (31) and, recently, by positional cloning from yeast artificial chromosome clones (32).

The aims of this study were to investigate the eventual structural differences of adducin subunits in MHS and MNS rats and their functional significance regarding blood pressure variation.

MATERIALS AND METHODS

Animal Procurement and Housing. All MNS and MHS rats were bred in our own facilities and maintained in conditions described elsewhere (9), in agreement with Directive 86/609/CEE of the Council of the European Community and Italian Law no. 116, 22/1/1992. The experimental F_2 population was produced as described in the text.

Blood Pressure Measurements. In the foundation colonies, selection for blood pressure levels was carried out each generation on awake animals restrained by wrapping lightly in a small cloth (33), using an indirect tail-cuff method. Measurements were made on a W + W BP recorder (Ugo Basile, Varese, Italy) with piezoelectric pickup.

For genetic analysis of the F_2 population, a cannula was inserted in the carotid artery of the rat under light halothane anesthesia and externalized at the back of the neck throughout a subcutaneous tunnel. The animals recovered within 3–5 min. Four hours later, the rats were connected by catheter to

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Abbreviations: MHS, Milan hypertensive strain; MNS, normotensive control.

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a Gould BS 3200 blood pressure recorder in unrestrained conditions. The blood pressure of each rat was recorded for 1 h as simultaneous systolic/diastolic every 3–4 min and the tracings were read at 1-min intervals. These values were averaged to give a single systolic or diastolic measurement for each animal.

Synthetic Peptide Sequences. Peptides had the following sequences: βR, VIAEKSRSPSTE; βQ, VIAEKSQSPSTE; αY, VEEAFYYIHNLV; αF, VEEAFFYIHNLV; S, RFARKGSLRQKNV.

Peptide Phosphorylation. *Protein kinase C.* The reaction mixtures contained 40 mM Hepes (pH 7.4), 8 mM $MgCl_2$, 0.8 mM $CaCl_2$, 0.5 mM EGTA, 40 μM [γ-^{32}P]ATP (4500 dpm/pmol), 80 μg of phosphatidylserine per ml, 20 μg of dioleoylphosphatidylcholine per ml, 200 μg of bovine serum albumin per ml, 0.8 mg of peptide (βR, βQ, or S) per ml and enzyme in a total vol of 65 μl. Incubations were carried out at 30°C for 30 min. Protein kinase C was partially purified from rat brain (34).

Protein kinase A. The reaction mixtures contained 100 mM Tris·HCl (pH 7.5), 12 mM $MgCl_2$, 100 μM [γ-^{32}P]ATP (5000 dpm/pmol), 250 μg of bovine serum albumin per ml, 0.1 mM dithiothreitol, 7.5% (vol/vol) glycerol, 0.8 mg of peptide (βR, βQ, or S) per ml, and cAMP-dependent protein kinase catalytic subunit (0.5 unit/ml) in a total vol of 50 μl. Incubations were carried out at 33°C for 1 h.

After incubation with either protein kinase C or the catalytic subunit of protein kinase A, excess ATP was eliminated by incubating 1/10th of the phosphorylated peptide mixtures with T4 polynucleotide kinase (200 units/ml) and a 24-mer oligonucleotide acceptor (1.5 mg/ml) in 50 mM Tris·HCl, pH 7.6/7 mM $MgCl_2$/3 mM dithiothreitol at 37°C for 15 min.

Tyrosine Protein Kinase. The reaction mixtures contained 100 mM Tris·HCl (pH 7.5), 5 mM $MgCl_2$, 20 μM sodium vanadate, 0.08 mg of poly(L-lysine) per ml, 20 μM [γ-^{32}P]ATP (4000 dpm/pmol), 200 μM peptide (αY and αF), and tyrosine protein kinase *lyn*-TPK (product of the *lyn* oncogene) purified from rat spleen (35) in a total vol of 50 μl. Incubations were carried out at 30°C for 15 min and stopped with 450 μl of 30% AcOH. Excess ATP was eliminated on Dowex 1X8 columns equilibrated in 30% AcOH.

Phosphopeptide Analysis. Peptides phosphorylated with protein kinase A, protein kinase C, or TPK-IIA tyrosine kinase were separated by ascending chromatography on cellulose-coated TLC plates, with the solvent system butanol/pyridine/acetic acid/water (15:10:3:12). The plates were dried and autoradiographed (36).

Genotyping Studies. Genomic DNAs from F_2 rats were extracted from the tails (37) and 50 ng of genomic DNA was used per reaction. To detect the allelic variation in the α-adducin sequence, amplifications were performed in a total vol of 30 μl with the following reagent concentrations: 200 μM dNTPs, 0.5 μM each primer, and 0.7 unit of *Taq* DNA polymerase (Perkin–Elmer/Cetus). PCRs were carried out for 29 cycles (30 sec at 94°C, 20 sec at 53°C, and 30 sec at 72°C preceded by an initial denaturation at 94°C for 3 min) in a GeneAmp PCR system 9600 (Perkin–Elmer). The primers used to amplify a specific fragment of 99 bp of the α-adducin gene were αF/Y1 (5′-GTTCTCATTCTCCGGAATCAC-3′) and αF/Y2 (5′-CTGGATCTCACATGCAACCAC-3′). The nucleotide sequence of rat α-adducin flanking the point mutation is 5′-GTG GAG GAG GCC TTC T(T/A)T TAT ATC CAC AAC CTT-3′ (from nucleotide 1000 to nucleotide 1032 of the full-length cDNA). The amplified DNAs were applied to duplicate nylon filter membranes (GeneScreen) and hybridized with allele-specific oligonucleotide probes. The ^{32}P-end-labeled oligonucleotides used as probes were αA (5′-GATATAATAGAAGGC-3′) and αT (5′-GATATAAAAGAAGGC-3′). Prehybridization and hybridization were performed in 5× standard saline citrate (SSC)/10× Denhardt's solution/0.1% SDS/100 μg of salmon sperm DNA per ml for 2 h at 45°C. The filters were washed twice in 5× SSC/0.1% SDS for 10 min at 45°C.

To detect the allelic variation in the β-adducin sequence, amplifications were carried out under the following conditions: 32 cycles of 50 sec at 94°C, 50 sec at 55°C, and 50 sec at 72°C, preceded by an initial denaturation at 94°C for 2 min. The primers used to amplify a specific fragment of 290 bp of the β-adducin gene were βQ/R1 (5′-AGGACCTTGTCAGATCCATC-3′) and βQ/R2 (5′-GAGCCCAGCACCTAAGGTCA-3′). The nucleotide sequence of rat β-adducin flanking the point mutation is 5′-ATC GCC GAG AAG AGC C(A/G)G AGT CCG TCT ACA GAG-3′ (from nucleotide 1756 to nucleotide 1788 of the full-length cDNA). The adenine to guanine transition results in a restriction fragment length polymorphism due to an additional *Hpa* II site in the guanine substitution. After the PCR, the samples were digested with *Hpa* II, ^{32}P-end-labeled, and submitted to electrophoresis in 12% nondenaturing acrylamide gel. The *Hpa* II polymorphism was visualized as a restriction fragment of 53 bp for the adenine and 46 bp for the guanine substitution.

Statistical Analysis. Results are expressed as means ± SEM. Statistical analyses were performed with SPSS (version 4) statistical software (SPSS, Chicago). Data were analyzed by one-way and two-way ANOVAs as appropriate. To take into account the possible effects of extreme observations, homogeneity of variances were always tested by Cochran's *C* and Bartlett Box *F* (data not shown) after having performed one-way ANOVA. The Kruskal–Wallis nonparametric test was also used. Finally, after one-way ANOVA, *a posteriori* multiple comparisons were performed using both Fisher's least significant difference test and Student's–Newman–Keuls test. Both tests gave essentially the same results for a *P* value of 0.05. Frequency distributions of alleles in the F_2 population were tested with χ^2 in comparison to the expected Mendelian transmission.

RESULTS

We have previously demonstrated a polymorphism in a β-adducin 63 cDNA isolated from MHS and MNS strains (30). A guanine to adenine transition resulted in an arginine (MHS) → glutamine (MNS) substitution at position 529 (R529Q). The presence of this polymorphism on the major alternative splicing β-adducin mRNA (β-adducin 97) was confirmed by specific PCR analysis of cDNAs synthesized

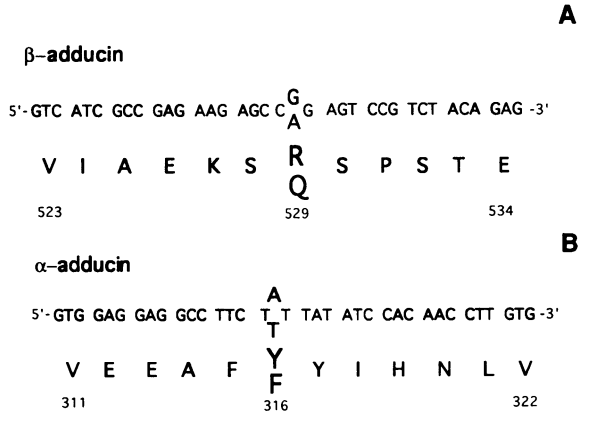

FIG. 1. Nucleotide and predicted amino acid sequences surrounding the mutation sites of adducin subunits in MHS and MNS rats. (*A*) Nucleotide sequence of β-adducin showed a G to A transition resulting in an arginine (MHS) to glutamine (MNS) substitution (R529Q). (*B*) Nucleotide sequence of α-adducin showed an A to T transversion resulting in a tyrosine (MHS) to phenylalanine (MNS) substitution (Y316F).

from β-adducin 97 coding sequences (Fig. 1*A*). Sequence analysis of the α-adducin chain has shown an adenine to thymine transversion, resulting in a tyrosine (MHS) → phenylalanine (MNS) substitution at position 316 (Y316F) (Fig. 1*B*).

The high state of inbreeding in both strains was confirmed by DNA fingerprinting analysis performed on 20 animals of each strain using the multilocus minisatellite probes 33.6 and 33.15 (38). No differences in band patterns were found within either strain. Furthermore, general homozygosity in MNS and MHS was tested by using 65 monolocus microsatellite markers, revealing only one segregating locus in MNS and none in MHS.

MHS and MNS Genotyping. When a large group of animals from each strain was analyzed for the alleles of the α- and β-adducin genes, all MHS rats were found to be homozygous $\alpha^Y\beta^R/\alpha^Y\beta^R$, while MNS rats were homozygous for α-adducin (α^F/α^F) but were segregating at the β-adducin locus for β^Q/β^Q, β^Q/β^R, β^R/β^R. The persistent heterozygosis for the β-adducin gene in MNS, despite the high inbreeding, could be explained by selection for low blood pressure, optimization of biological fitness, or a recent mutation. The first possibility was tested by measuring weekly the systolic blood pressure of 38 rats of the MNS foundation colony, using the same method as for selection. When the rats were subdivided according to their β-adducin genotypes, the MNS β^Q/β^R were found to have a systolic blood pressure (128.7 ± 1.6 mmHg; $n = 17$) lower than the other two genotypes (β^R/β^R, 134.8 ± 1.8 mmHg; $n = 12$; β^Q/β^Q, 133.2 ± 1.4 mmHg; $n = 9$; $P = 0.003$). Moreover, MNS was separated into two coisogenic substrains with β^R/β^R and β^Q/β^Q genotypes and F_1 hybrids were obtained.

Again, the blood pressure of the heterozygotes (F_1 hybrids) was lower than that of the two homozygous groups (β^Q/β^Q, 134.5 ± 0.5 mmHg; $n = 67$; β^Q/β^R, 131.9 ± 0.5 mmHg; $n = 59$; β^R/β^R, 134.5 ± 0.8 mmHg; $n = 32$). A hypothetical influence of biological fitness was tested by measuring reproduction, body growth, and sex ratio in the three populations of rats (β^Q/β^Q, β^Q/β^R, β^R/β^R), but no differences were found. The first immunological heterogeneity of adducin in MNS was detected 5 years ago (25) and has persisted for 15 generations of sister–brother mating.

Differential Phosphorylation of αF–αY and βQ–βR Peptides. The region comprising residue 529 of β-adducin was compared to a data base of functional domains (39) and homologies were found to various domains involved in phosphorylation. The best similarity was found to a basic nuclear protein phosphorylation site described in the winter flounder (40). Furthermore, the sequence around residue 316 of α-adducin was also a possible tyrosine kinase substrate site. The changes in amino acids determined by the variations in α- and β-adducin suggest the possibility of corresponding changes in the extent of phosphorylation in that region of the molecule. The substitution of R for Q in β-adducin is likely to have an effect on the phosphorylation of S residues in the vicinity, and the change of Y to F deletes a putative phosphorylation site in α-adducin. Each of these possibilities was examined by studying the phosphorylation of synthetic peptides corresponding to the relevant region of both adducin subunits (Fig. 2). The β-adducin peptides βR and βQ were not phosphorylated by protein kinase C in conditions leading to strong phosphorylation of a synthetic substrate (peptide S). On the contrary, peptide βR was phosphorylated much more than peptide βQ by the catalytic subunit of the cAMP-dependent protein kinase. Similarly, peptide αY but not peptide αF was phosphorylated by tyrosine kinase ($^{32}P_i$ incorporation measured by scraping the cellulose and counting yielded 52,540 cpm in βR and 175 cpm in βQ, 45,050 cpm in αY and 512 cpm in αF). This supports the hypothesis that the presence of the β^Q and α^F alleles results in a decreased phosphorylation

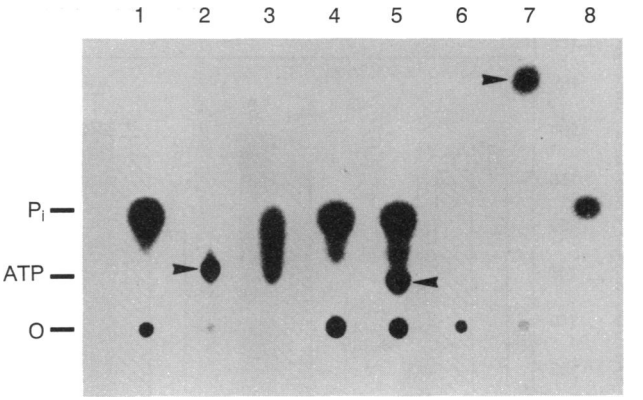

FIG. 2. Phosphorylation of adducin peptides. Analysis by TLC. Aliquots (1–4 μl) from incubation mixtures of the different peptides with the catalytic subunit of protein kinase A and TPK-IIA were loaded on cellulose-coated plates, run, and autoradiographed as described in *Materials and Methods*. Lanes: 1, control (no peptides); 2, peptide S (protein kinase A and C substrate); 3, [γ-^{32}P]ATP; 4, peptide βQ; 5, peptide βR; 6, peptide αF; 7, peptide αY; 8, ^{32}P. Arrowheads in lanes 2, 5, and 7 indicate positions of phosphorylated S, βR, and αY, respectively.

status of α- and β-adducin *in vivo*. The functional role of these phosphorylations is difficult to test *in vivo* because of multiple phosphorylation sites present in both α- and β-adducin. However, by crossing the rat strains, phenotypic studies were possible, which are described below.

MNS $\alpha^F\beta^Q/\alpha^F\beta^Q$ × MHS $\alpha^Y\beta^R/\alpha^Y\beta^R$ Crosses. An F_1 generation was produced by reciprocally mating parental strain MNS ($\alpha^F\beta^Q/\alpha^F\beta^Q$) and MHS ($\alpha^Y\beta^R/\alpha^Y\beta^R$) rats. The average systolic blood pressures, measured in both sexes, revealed no maternal or paternal effects in F_1 hybrids, which were then intercrossed. The resulting F_2 population consisted of equal numbers of offspring from each reciprocal outcross and the frequency distributions of all alleles considered were not significantly different from the Mendelian expectations for both sexes ($\chi^2 \leq 1.5$; $P \geq 0.47$). Blood pressure in the F_2 rats was measured by a catheter introduced in the carotid artery so that systolic and diastolic pressure could be recorded. Also, the blood pressure of 10 rats from each parental strain was measured as in F_2 rats on a blind basis. The corresponding average values of systolic and diastolic blood pressures were as follows: MHS, 167.7 ± 2.2 and 116.2 ± 1.7 mmHg; MNS, 139.6 ± 2.3 and 98.7 ± 2.6 mmHg. Fig. 3 shows the average values of the diastolic and systolic blood pressure in the adducin genotype cohorts. The average heart rate was identical in all groups (data not shown). Taken separately on the undefined segregating F_2 background, α^Y/α^Y alleles significantly contributed to an increase in systolic blood pressure over α^F/α^F alleles by ≈8 mmHg—i.e., 28% of the difference between the parental strains.

No difference in blood pressure was seen when the three β-adducin genotypes of all F_2 cohorts were compared. However, female β^Q/β^R heterozygotes had a lower blood pressure than the two homozygous groups.

The blood pressure differences between $\alpha^F\beta^Q/\alpha^F\beta^Q$ and $\alpha^Y\beta^R/\alpha^Y\beta^R$ were small (156.4 ± 2.1 and 161.3 ± 2.6 mmHg, respectively) and of borderline statistical significance ($P < 0.1$). MHS and MNS are the result of selection for high and low blood pressure and several genetic differences are likely to contribute to the phenotype. The adducin polymorphisms are only two of the differences and their influence on blood pressure values may be attenuated on the genetic background of the F_2 population.

Fig. 3 shows that the difference in blood pressure between the adducin α^Y/α^Y (MHS type) and α^F/α^F (MNS type) genotypes depends on the associated β alleles. In fact, this

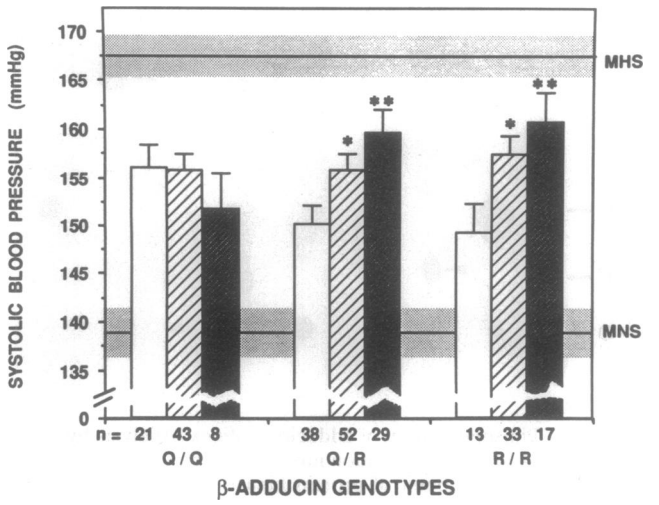

FIG. 3. Systolic blood pressure of F_2 population in different adducin genotype cohorts ($\alpha^F\beta^Q/\alpha^F\beta^Q$, MNS genotype; $\alpha^Y\beta^R/\alpha^Y\beta^R$, MHS genotype). Systolic blood pressure (mmHg) was measured by a catheter inserted in the carotid artery. Results are expressed as means ± SEM. *A posteriori* multiple comparison tests for a P value of 0.05 or better were performed. *, Significantly different ($P < 0.05$) from α-adducin genotype F/F; **, significantly different ($P < 0.01$) from α-adducin genotype F/F. Mean systolic blood pressure (horizontal solid lines) ± SEM (stippled areas) of 10 rats of each parental strain was also included for appropriate comparison. Open bars, α-adducin genotype F/F; light hatched bars, α-adducin genotype F/Y; dark hatched bars, α-adducin genotype Y/Y.

difference is 12 mmHg—i.e., 43% of the systolic blood pressure difference between parental strains—in the presence of the β^R/β^R genotype (MHS type). In contrast, the difference is blunted in the presence of β^Q/β^Q (MNS type).

Taking into account the four possible genotypes for the homozygotes ($\alpha^Y\beta^Q/\alpha^Y\beta^Q$, $\alpha^Y\beta^R/\alpha^Y\beta^R$, $\alpha^F\beta^Q/\alpha^F\beta^Q$, $\alpha^F\beta^R/\alpha^F\beta^R$), two-way ANOVA indicated a significant interaction between the α and β genotypes for the blood pressure levels considered (significance of the interaction for systolic blood pressure, $P = 0.005$; for diastolic blood pressure, $P = 0.008$).

DISCUSSION

This study shows that adducin polymorphism is associated with blood pressure variations in the Milan rats. α^Y/α^Y MHS α-adducin alleles cosegregate with a significant increment in blood pressure and, interestingly, this effect is modulated by the associated β-adducin alleles. A major drawback of F_2 cosegregation studies (4, 41) is the impossibility of differentiating between the effect of the candidate gene and that of one closely linked to it. Our results show that in the MNS parental strain, selected for low blood pressure through 85 generations of inbreeding and whose homozygosity has been demonstrated, there is a selective advantage for heterozygotes at the β-adducin locus as these have a lower blood pressure than either homozygote. This supports the role of adducin polymorphism in the process of selection for low blood pressure in MNS. The lower blood pressure of the heterozygotes was confirmed when the two coisogenic substrains β^R/β^R and β^Q/β^Q were mated to obtain β^Q/β^R hybrids. These two coisogenic MNS substrains, which should differ only by β-adducin (β^R/β^R or β^Q/β^Q), constitute a unique model comparable to a homologous gene replacement and will be used to ascertain the interactions between α- and β-adducin subunits. The contribution of these genes to determining blood pressure levels is likely to depend on the assembly of the adducin heterodimer within the cell. It is highly improbable that the pressor effect is due to other genes closely linked to those of the adducin subunits because the adducin genes lie on different chromosomes and also because of the high degree of homozygosity of the strains used.

Adducin presents structural and functional similarities to the MARCKS proteins, which play an important role in the coupling of extracellular signals to the interactions between actin cytoskeleton and the cell membrane through phosphorylation events (29). In fact, α- and β-adducin have been shown to be extensively phosphorylated *in vivo* (42), although the precise residues involved are not known. The *in vitro* phosphorylation of the adducin synthetic peptides 311–322 (α^Y and α^F) and 523–534 (β^R and β^Q) has shown that there is the distinct possibility that the $\beta^R \rightarrow \beta^Q$ and $\alpha^Y \rightarrow \alpha^F$ variations will change the phosphorylation status of adducin and hence influence its cytoskeletal interactions and functions (27). Amino acid substitutions within the two adducin subunits may affect their assembly into stable heterodimers or the dimer–tetramer equilibrium and, in consequence, actin–spectrin (or fodrin) interactions (31). Thus, variation in the tension and strength of the resulting membrane skeleton network may cause changes in cell volume or membrane function. In fact, MHS erythrocyte resealed ghosts have a smaller volume and a lower K_m for internal Na of Na/K cotransport than those of MNS. The difference in K_m is not observed in inside-out vesicles deprived of membrane skeleton (14, 23, 24). These cell membrane alterations may represent the link between adducin point mutations and the cellular dysfunction responsible for hypertension in MHS. The hypothesis that membrane skeleton proteins regulate cell volume and ion transport is also supported by several previous findings (43–46).

As pointed out in the Introduction, a long series of findings supported the pressor role of the faster Na transport across the cell membrane and the role of membrane skeleton in determining the difference between MHS and MNS. Adducin was chosen for genetic studies because it was detected as the only membrane skeleton protein eliciting an immunological response in MHS–MNS cross-immunization experiments (25). However, this finding alone does not prove an involvement of adducin in membrane Na transport and, at present, we do not have data *in vivo* regarding the relationship between structure and function of adducin and the mechanism of its pressor effect.

Adducin polymorphisms account for only a portion of the blood pressure differences between MHS and MNS. Therefore, other genes may be involved, as suggested by the genetic analysis we carried out on classical genetic crosses (33). It could be hypothesized that a mutation that produced an increase in blood pressure happened in an α^Y/β^R background. The α^F/β^Q alleles would have been favored during the selection for low blood pressure in the MNS, as they compensate for the original mutation. This work demonstrates an interaction between two independently segregating genes determining the expression of a single protein that is involved in inheritance of primary hypertension. Considering the many similarities in kidney and cellular dysfunctions between MHS and subsets of patients with primary hypertension (10, 20–22) and the very high degree of sequence homology between rat and human adducin (data not shown), it is possible that these molecular mechanisms are also relevant to humans.

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1. Oparil, S. (1988) in *Cecil Textbook of Medicine*, eds. Wyngaar-

den, J. B., Smith, L. H. & Bennett, J. C. (Saunders, Philadelphia), pp. 253–269.
2. Folkow, B. (1982) *Physiol. Rev.* **62,** 347–504.
3. Rapp, J. P., Wang, S. M. & Dene, H. (1989) *Am. J. Hypertens.* **3,** 391–396.
4. Kurtz, T. W., Simonet, L., Kabra, P. M., Wolfe, S., Chan, L. & Hjelle, B. L. (1990) *J. Clin. Invest.* **85,** 1328–1332.
5. Hilbert, P., Lindpaintner, K., Beckmann, J. S., Serikawa, T., Soubrier, F., Dubay, C., Cartwright, P., De Gouyon, B., Julier, C., Takahasi, S., Vincent, M., Ganten, D., Georges, M. & Lathrop, G. M. (1991) *Nature (London)* **353,** 521–529.
6. Jacob, H. J., Lindpaintner, K., Lincoln, S. E., Kusumi, K., Bunker, R. K., Mao, Y.-P., Ganten, D., Dzav, V. J. & Lander, E. S. (1991) *Cell* **67,** 213–224.
7. Dubay, C., Vincent, M., Samani, N. J., Hilbert, P., Kaiser, M. A., Beressi, J.-P., Kotelevtsev, Y., Beckmann, J. S., Soubrier, F., Sassard, J. & Lathrop, G. M. (1993) *Nature Genet.* **3,** 354–357.
8. Cicila, G. T., Rapp, J. P., Wang, J. M., St. Lezin, E., Ng, S. C. & Kurtz, T. W. (1993) *Nature Genet.* **3,** 346–353.
9. Bianchi, G., Ferrari, P. & Barber, R. B. (1984) in *Handbook of Hypertension: Experimental and Genetic Models of Hypertension*, ed. de Jong, W. (Elsevier, Amsterdam), pp. 328–349.
10. Bianchi, G., Ferrari, P. & Barber, B. R. (1990) in *Hypertension: Pathophysiology, Diagnosis and Management*, eds. Laragh, J. H. & Brenner, B. M. (Raven, New York), pp. 901–922.
11. Bianchi, G., Fox, U., Di Francesco, G. F., Giovanetti, A. M. & Pagetti, D. (1974) *Clin. Sci. Mol. Med.* **47,** 435–448.
12. Salvati, P., Ferrario, R. G., Parenti, P. & Bianchi, G. (1987) *J. Hypertens.* **5,** 31–38.
13. Bianchi, G., Baer, P. G., Fox, U., Duzzi, L., Pagetti, D. & Giovanetti, A. M. (1975) *Circ. Res.* **36/37,** Suppl. 1, 153–161.
14. Ferrari, P., Ferrandi, M., Torielli, L., Canessa, M. & Bianchi, G. (1987) *J. Hypertens.* **5,** 199–206.
15. Ferrari, P., Nussdorfer, G., Torielli, L., Salvati, P., Tripodi, M. G., Niutta, E. & Bianchi, G. (1987) in *The Early Pathogenesis of Primary Hypertension*, eds. Hofman, A., Grobbee, D. E. & Schalekamp, M. A. D. H. (Elsevier, Amsterdam), pp. 63–67.
16. Parenti, P., Hanozet, G. & Bianchi, G. (1986) *Hypertension* **8,** 932–939.
17. Ferrandi, M., Salardi, S., Parenti, P., Ferrari, P., Bianchi, G., Braw, R. & Karlish, S. J. D. (1990) *Biochim. Biophys. Acta* **1021,** 13–20.
18. Bianchi, G., Ferrari, P., Trizio, D., Ferrandi, M., Torielli, L., Barber, B. R. & Polli, E. (1985) *Hypertension* **7,** 319–325.
19. Ferrari, P., Barber, B. R., Torielli, L., Ferrandi, M., Salardi, S. & Bianchi, G. (1987) *Hypertension* **10,** 132–136.
20. Cusi, D., Fossali, E., Piazza, A., Tripodi, G., Barlassina, C., Pozzoli, E., Vezzoli, G., Stella, P., Soldati, L. & Bianchi, G. (1991) *Am. J. Hypertens.* **4,** 725–734.
21. Bianchi, G., Cusi, D., Barlassina, C., Lupi, G. P., Ferrari, P., Picotti, G. B., Gatti, M. & Polli, E. (1983) *Kidney Int.* **23,** 870–875.
22. Guidi, E., Bianchi, G., Rivolta, E., Ponticelli, C., Quarto Di Palo, F., Minetti, L. & Polli, E. (1985) *Nephron* **41,** 14–21.
23. Ferrari, P., Torielli, L., Cirillo, M., Salardi, S. & Bianchi, G. (1991) *J. Hypertens.* **9,** 703–711.
24. Ferrari, P., Torielli, L., Salardi, S., Rizzo, A. & Bianchi, G. (1992) *Biochim. Biophys. Acta* **1111,** 111–119.
25. Salardi, S., Saccardo, B., Borsani, G., Modica, R., Ferrandi, M., Tripodi, M. G., Soria, M., Ferrari, P., Baralle, F. E., Sidoli, A. & Bianchi, G. (1989) *Am. J. Hypertens.* **2,** 229–237.
26. Gardner, K. & Bennett, V. (1986) *J. Biol. Chem.* **261,** 1339–1348.
27. Kaiser, H. W., O'Keefe, E. & Bennet, V. (1989) *J. Cell Biol.* **109,** 557–569.
28. Mische, S. M., Mooseker, M. S. & Morrow, J. S. (1987) *J. Cell Biol.* **105,** 2837–2845.
29. Aderem, A. (1992) *Cell* **71,** 713–716.
30. Tripodi, G., Piscone, A., Borsani, G., Tisminetzky, S., Salardi, S., Sidoli, A., James, P., Pongor, S., Bianchi, G. & Baralle, F. E. (1991) *Biochem. Biophys. Res. Commun.* **177,** 939–947.
31. Joshi, R., Gilligan, D. M., Otto, E., McLaughlin, T. & Bennet, V. (1991) *J. Cell Biol.* **115,** 665–675.
32. Taylor, S. A. M., Snell, R. G., Buckler, A., Ambrose, C., Duyao, M., Church, D., Lin, C. S., Altherr, M., Bates, G. P., Groot, N., Barnes, G., Shaw, D. J., Lehrach, H., Wasmuth, J. J., Harper, P. S., Housman, D. E., MacDonald, M. E. & Gusella, J. F. (1992) *Nature Genet.* **2,** 223–227.
33. Schlager, G., Barber, B. R. & Bianchi, G. (1986) *Can. J. Genet. Cytol.* **28,** 967–970.
34. House, C., Wettenhall, R. E. H. & Kempt, B. E. (1987) *J. Biol. Chem.* **262,** 772–777.
35. Brunati, A. M., Donella-Deana, A., Marchiori, F., Borin, G., Fischer, S. & Pinna, L. A. (1991) *Biochim. Biophys. Acta* **1091,** 123–126.
36. Alper, S. L., Palfrey, H. C., DeRiemer, S. A. & Greengard, P. (1980) *J. Biol. Chem.* **255,** 11029–11039.
37. Laird, P. W., Zijderveld, A., Linders, K., Jaenisch, R. & Berns, A. (1991) *Nucleic Acids Res.* **19,** 4293.
38. Jeffreys, A. J., Wilson, V. & Thein, S. L. (1985) *Nature (London)* **314,** 67–73.
39. Pongor, S., Skerl, V., Cserzo, M., Hatsagi, Z., Simon, G. & Bevilacqua, V. (1993) *Nucleic Acids Res.* **21,** 3111–3115.
40. Kennedy, B. P. & Davies, P. L. (1985) *J. Biol. Chem.* **260,** 4338–4344.
41. Rapp, J. P. & Wang, S. E. (1990) in *Hypertension: Pathophysiology, Diagnosis and Management*, eds. Laragh, J. H. & Brenner, B. M. (Raven, New York), pp. 955–964.
42. Waseem, A. & Palfrey, H. C. (1988) *Eur. J. Biochem.* **178,** 563–573.
43. Spring, K. R. & Hoffmann, E. K. (1992) in *The Kidney: Physiology and Pathophysiology*, eds. Seldin, D. W. & Giebish, G. (Raven, New York), 2nd Ed., pp. 147–170.
44. Sarkadi, B. & Parker, J. C. (1991) *Biochim. Biophys. Acta* **1071,** 407–427.
45. Nelson, W. J. (1992) *Science* **258,** 948–955.
46. Cantiello, H. F., Prat, A. G., Bonventre, J. V., Cunningham, C. C., Hartwig, J. H. & Ausiello, D. A. (1993) *J. Biol. Chem.* **268,** 4596–4599.