The Milan Hypertensive Rat as a Model for Studying Cation Transport Abnormality in Genetic Hypertension

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SUMMARY Environmental factors, genetic polymorphisms, and different experimental designs have been the main impediments to evaluating a genetic association between cell membrane cation transport abnormalities and human essential or genetic hypertension. We review the results obtained in the Milan hypertensive strain of rats (MHS) and in its appropriate control normotensive strain (MNS) to illustrate our approach to defining the role of cation transport abnormality in a type of genetic hypertension. Before the development of a difference in blood pressure between the two strains, the comparison of kidney and erythrocyte functions showed that MHS had an increased glomerular filtration rate and urinary output, and lower plasma renin and urine osmolality. Kidney cross-transplantation between the strains showed that hypertension is transplanted with the kidney. Proximal tubular cell volume and sodium content were lower in MHS while sodium transport across the brush border membrane vesicles of MHS was faster. Erythrocytes in MHS were smaller and had lower sodium concentration, and Na\(^+\)-K\(^+\) cotransport and passive permeability were faster. The differences in volume, sodium content, and Na\(^+\)-K\(^+\) cotransport between erythrocytes of the two strains persisted after transplantation of bone marrow to irradiated F\(_2\) (MHS x MNS) hybrids. Moreover, in normal segregating F\(_2\) hybrid populations there was a positive correlation between blood pressure and Na\(^+\)-K\(^+\) cotransport. These results suggest a genetic and functional link in MHS between cell membrane cation transport abnormalities and hypertension. Thus erythrocyte cell membrane may be used for approaching the problem of defining the genetically determined molecular mechanism underlying the development of a type of essential hypertension.

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KEY WORDS • rat model • genetic hypertension • Milan hypertensive rats • Milan normotensive rats • erythrocyte ion transport

DIFFERENT types of alterations in cation transport across cell membranes have been found to occur in humans with essential hypertension,\(^1\) and despite many efforts, the relationship between these alterations and arterial hypertension is far from clear. Environmental factors, genetic polymorphisms, and the difficulties of realizing the right experimental design to study the relationships among these factors are the main causes of this confusion. An animal model with genetic hypertension may represent the ideal tool for studying these problems and for providing the theoretical background of knowledge for approaching them in the human disease.

The history of hypertension clearly shows the usefulness of animal models for understanding the human disease: for example, renal artery constriction in the dog\(^3\) and in the rat.\(^4\) In fact, only after having demonstrated a clear cause-effect relationship between renal artery constriction and hypertension in these animal models was it possible to set up in humans a suitable diagnostic approach for separating this secondary form of hypertension from the whole group of essential hypertension.
The hypothetical cause-effect relationship between alterations in cation transport and hypertension in animal models is much more difficult to demonstrate or exclude for a number of reasons, however. First, because of the very complex network of feedback mechanisms regulating the biochemical functions of cells, any genetic alteration of an individual cellular constituent produces other biochemical changes aimed at compensating for the primary genetic alteration. Thus the whole biochemical machinery of the cell is shifted to a new equilibrium. Second, alteration in cell function causes alterations in organ function, and both produce in other cells or organs additional changes in an attempt to compensate for the primary alterations at whole-body level. This again produces a shift in the balance between different feedback mechanisms, thus altering humoral, nervous, or other factors, and possibly also modifying and masking the primary alteration.

For these reasons the alterations in cation transport described thus far in hypertension may be considered as 1) the direct phenotypic expression of the genetic abnormality causing hypertension; 2) a secondary event that represents just a part of the complex readjustment of cell biochemical machinery and is caused by a genetic abnormality affecting some other biochemical mechanism; 3) the effect, through independent mechanisms of linkage, between genes affecting ion transport and blood pressure without any cause-effect relationship between them; or 4) an epiphenomenon not relevant to blood pressure control.

To distinguish among these possibilities, our investigations had the following basic goals:

1. To establish whether a given difference in organ function between a spontaneous animal model of hypertension and its appropriate control is responsible for the difference in blood pressure, and whether that difference in function is due to an intrinsic property of the organ or caused by some secondary humoral, hemodynamic, or neural factor.
2. To evaluate whether a given cellular abnormality is genetically determined in the stem cells or is secondary to some extracellular factor.
3. To evaluate possible genetic associations between the cell abnormality and the development of hypertension in the segregating F1 population obtained by crossing the F1 (hypertensive X control) hybrids.
4. To assess possible physiological-biochemical links between the cell abnormality studied at Points 2 and 3 and the organ dysfunction responsible for hypertension studied at Point 1.

Once having established a cause-effect relationship between a given cell abnormality and the development of hypertension by the studies at Points 1, 2, 3, and 4, the most difficult task was to evaluate among the many biochemical alterations characterizing the cell abnormality which one is primarily linked to the genetic alteration and which is due to secondary readjustment. In this context the role of alteration in cation transport across the cell membrane may be properly established.

The results so far obtained studying the Milan hypertensive strain of rats (MHS) and its control normotensive strain (MNS) are reviewed with respect to these considerations.

Organ Dysfunction

For a number of reasons discussed in detail elsewhere, we especially studied the role of the kidneys and showed that during the prehypertensive stage, the kidneys of MHS had a higher glomerular filtration rate (GFR) and urinary output, and decreased urine osmolality and renin secretion. These differences were found by studying both the whole animal in the conscious state and the isolated perfused kidney in vitro. Moreover, sodium transport across the membrane of brush border vesicles, studied in vitro, was higher in MHS at the prehypertensive stage. Most of these differences tended to disappear as hypertension developed in MHS. Studies of sodium balance showed renal retention of sodium in MHS. Proximal tubular cell volume and sodium concentration were lower in MHS, but no difference between the two strains was found in distal tubular cells or in liver cells.

Kidney cross-transplantation between MHS and MNS showed that hypertension can be "transplanted" with the kidney. Therefore the renal dysfunction found in MHS at the prehypertensive stage is, in some way, linked to the cause of hypertension. In our view, the hypothesis that best reconciles the increase in GFR, the decrease in renin secretion, and the reduced concentrating ability of the kidneys with the transplantation results and renal retention of sodium during the development of hypertension in MHS, is that postulated by faster sodium reabsorption across the proximal tubular cell (see References 13, 15, 24, and 25 for a full discussion on this point). This hypothesis, implying a cell defect, also may be tested by studying blood cells according to the indications given at Points 2, 3, and 4 on the assumption that the genetic abnormality in sodium transport across the proximal tubular cells may also be found in blood cells.

Cell Dysfunction

In the prehypertensive stage, cell volume and sodium concentration in MHS erythrocytes were smaller while the Na+/K+ cotransport was faster. No difference between the two strains was found in Na+/K+ pump activity.

To test whether these abnormalities are genetically determined within the stem cells or secondary to some extracellular factor, we transplanted bone marrow from MHS and MNS to irradiated F1 hybrids. The control F1 hybrids, which did not receive exogenous bone marrow, died within 15 days after irradiation. The F1 rats that received bone marrow from MHS or MNS survived in good health for many months, and their red blood cells showed the same characteristics as those of the bone marrow donors. This demonstrates that the biochemical mechanisms responsible for these cell abnormalities are genetically determined within the stem cell itself.

In 150-day-old animals, when hypertension in MHS was fully developed, Na+/K+ pump was lower in MHS while Na+/K+ cotransport became equal in the two strains. This indicates that extracellular factors,
probably humoral, affect cation transport across the erythrocyte membranes and mask the functional pattern that is genetically determined in the stem cells.

**Genetic Association Between Cell Dysfunction and Hypertension**

The segregating F2 generation that is obtained by crossing F1 (MHS X MNS) hybrids is suitable for studying genetic associations between phenotypic traits. In particular, we measured blood pressure and erythrocyte Na\(^+\)-K\(^+\) cotransport in individual rats of the F2 generation. The results showed a positive and statistically significant \((r = 0.52, p < 0.001)\) correlation between the two values.

This correlation may be due to either pleiotropic effects or to linkage of single genes or groups of genes that regulate erythrocyte characteristics and blood pressure. At present we cannot distinguish between the possibilities, and genetic studies are being conducted to answer this question.

**Biochemical-Physiological Link Between Kidney Dysfunction and Erythrocyte Dysfunction**

As mentioned at Point 1, the results so far available on the kidney dysfunction responsible for the development of hypertension in MHS are consistent with the hypothesis of a primary change in proximal tubular reabsorption as the cause of both conditions. Moreover, direct measurement of cell volume and sodium concentration in proximal tubular cells showed a reduction of these two factors in MHS, while no difference was seen between the two strains when distal tubular cells or liver cells were compared. Furthermore, sodium transport across the brush border membrane vesicles was faster in MHS. Considering Points 2 and 3, it is clear that from the functional point of view, the abnormalities in MHS erythrocytes and proximal tubular cells have many similarities, even though the underlying molecular mechanisms remain to be elucidated. This biochemical-physiological link between erythrocytes and tubular cells favors the hypothesis that the genetic association described at Point 3 is due to a pleiotropic effect of the same gene or genes and not to linkage.

**Future Directions for Clarifying Genetic Molecular Mechanisms Underlying Cellular Functional Changes**

In addition to the differences in MHS and MNS cell volumes, and in sodium concentration and transport across the plasma membrane at proximal tubular cell or erythrocyte levels, the following differences have been found between MHS and MNS erythrocytes:

1. Decreased cell surface in MHS, calculated from the volume of the cells at the maximum swelling point in hypotonic solution.
2. A different ratio between some phospholipid and fatty acids in the membrane of the MHS red cells (S. Salardi et al., unpublished data, 1986).
3. Decreased activity of the calmodulin-stimulated calcium adenosine triphosphatase at maximum rate of enzymatic reaction in MHS.
4. Decreased microviscosity of the MHS red cell membrane, measured by the fluorescence-emission technique using the dye 1,6-diphenyl-1,3,5-hexatriene (A. Azzi, personal communication, 1985).

In this context it is not possible to say whether the alteration in cation transport is the direct phenotypic expression of the genetic abnormality responsible for hypertension or a secondary change in cell function that occurs to achieve the new equilibrium indispensable to cell survival. This second possibility does not exclude a physiological link between the alteration in cation transport and hypertension. However, the answer to this question is crucial for the definition of a primary molecular mechanism through which the genetic message for hypertension initiates the biochemical sequence of events leading to the overall cell abnormality, and thus to organ dysfunction, responsible for the increase in blood pressure. How should this question be approached?

Considering that we were dealing with genetically determined biochemical abnormalities, the most advanced approach to this question is to search for a structural difference between the proteins of MHS and MNS cells, and if that is found, to search for the correspondence between structural and functional differences. In fact, among the many biochemical functional differences between MHS and MNS cells, those that are linked to structural differences are likely to be primary and genetically determined. We are pursuing this approach by using immunological and molecular biology techniques to study the cell membrane proteins of MHS and MNS.

Along this line we recently obtained an interesting result by performing a cross-immunization of MHS with ghosts from MNS and vice versa. In this experiment only MHS rats were able to raise antibodies against a 105-kDa protein located in erythrocyte membrane skeleton. This immunological difference might underlie a structural difference in this protein between the two strains. Another approach is to modify one factor at a time and observe any changes occurring in cation transport.

For example, if we abolish the difference in erythrocyte volumes between MHS and MNS by hypotonic swelling, the difference in Na\(^+\)-K\(^+\) cotransport also disappears. Moreover, the smaller volume of MHS erythrocytes is maintained in their resealed ghosts, indicating that most likely a structural alteration of cell membrane and not a cytoplasmic factor is responsible for this defect in MHS. Finally, the Na\(^+\)-K\(^+\) cotransport recently measured by us in erythrocyte inside-out vesicles (which are deprived of membrane skeleton) did not show any difference in the two strains. These findings further support the hypothesis that a different or absent membrane skeleton protein is involved in the cell volume and ion transport alterations of MHS.

Several investigators have studied the Na\(^+\)-K\(^+\) fluxes in erythrocytes of other strains of genetically hypertensive rats. Unfortunately, different methodologies were used in different laboratories, and therefore comparison of the result is difficult. In all the genetically hypertensive strains studied, however, an
abnormality of the cell ion handling was also found before the development of hypertension, as in spontaneously hypertensive rats (SHR) and MHS. Since in hypertensive humans subpopulations with different alterations of red blood cell ion transport have been recognized, it is conceivable that different genetically hypertensive strains may represent a model for these subpopulations.

In conclusion, we believe that a fruitful approach to understanding the role in arterial hypertension of cation transport abnormalities is to consider them as part of an overall cell abnormality. Taking advantage of the fact that most strains of animals with genetic hypertension are fully inbred and therefore homozygous at practically all loci, a combined approach using genetic analysis and immunochemistry techniques should allow us to demonstrate the existence of even minor differences in protein structure; and then the sequence of events going from a protein structural abnormality to the overall cell abnormality might be clarified. It is within this context that the role of cation transport abnormalities may be evaluated properly. Finally, the identification of a specific protein defect would allow the use of modern, molecular biological methods to detect the gene or genes of interest.

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