GENETIC ANALYSIS OF INHERITANCE OF RATES OF Na^+,K^+-COTRANSPORT, CALCIUM CONCENTRATION IN ERYTHROCYTES, AND BLOOD PRESSURE OF F_2 HYBRIDS OF SPONTANEOUSLY HYPERTENSIVE AND NORMOTENSIVE RATS

Yu. V. Kotelevtsev, S. N. Orlov, N. I. Pokudin, V. M. Agnaev, and Yu. V. Postnov

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In spontaneously hypertensive rats abnormalities are found in the membrane transport systems of the erythrocytes for monovalent cations and calcium [2]. To study the problem whether the changes found are of fundamental importance in the process of development of hypertension, analysis of the linkage of their inheritance with the rise of blood pressure (BP) is necessary.

In this investigation inheritance of the rates of Na^+,K^+-cotransport, measured as the furosemide-sensitive component of the ^{86}Rb inflow, the ^{45}Ca concentration in the erythrocytes in the presence of orthovanadate, and BP were analyzed in second generation hybrids (F_2) of spontaneously hypertensive Kyoto-Wistar rats (SHR) and normotensive Kyoto-Wistar rats (WKY) of the control line.

EXPERIMENTAL METHOD

Altogether 41 F_2 hybrids were used (male rats aged 16 weeks and weighing 320-360 g). The SHR and WKY rats and also the F_1 hybrids were mated at the age of 15-17 weeks. The systolic BP (BP_1) was measured in unanesthetized animals by a plethysmographic method, the appearance of pulsation in the caudal artery being recorded when the air pressure in the cuff was lowered. Pulsation and air pressure in the cuff were recorded on a polygraph (Mingograf 34, Siemens-Elema, Sweden) by means of an MPP-3C photoelectric transducer (Nihon Kohden, Japan) and an LS22 electromanometer (Boucke-Brecht, West Germany). Before the measurements the animals were warmed for 15 min at 37°C. The measurements were made in the morning and repeated several times until 10% convergence of the measured value was obtained. The mean value of BP_1 was calculated on the basis of data obtained during measurements for 5 days. Before the animals were sacrificed, their BP also was measured by the direct method (BP_2). The animals were anesthetized with ether for 4.5 min, after which, through a laparotomy incision, a needle connected through a catheter, filled with physiological saline, to a pressure transducer 746 (Siemens-Elema) was inserted into the bifurcation of the abdominal aorta. Pressure was recorded on the polygraph for 4 min. BP_2 was determined as the sum of the systolic and diastolic pressures, divided by 2. Methods of taking blood, obtaining erythrocytes, and determining the rate of inflow of ^{86}Rb and accumulation of ^{45}Ca in the presence of orthovanadate were described by the writers previously [1, 3]. Correlation analysis was carried out by means of standard programs on a microcomputer (Kontron MOP-Videoplain).

A continuous distribution by pressure and by values of Na^+,K^+-cotransport and calcium concentration was found in the F_2 hybrids. The limits of distribution coincided with the corresponding values for the original SHR and WKY lines. It will be clear from Fig. 1 that positive correlation was found between the rate of Na^+,K^+-cotransport and the value of BP_2 ($r = 0.509$; $p < 0.001$). The quantity of calcium accumulated by erythrocytes in the presence of orthovanadate correlates with the value of BP_1 ($r = 0.32$; $p < 0.05$) but does not correlate with the value of BP_2, measured under anesthesia. The coefficient of correlation rose to 0.45 ($p < 0.005$) when the calcium concentration was plotted against ΔBP ($BP_1 - BP_2$) (Fig. 2). Thus the calcium concentration in the erythrocytes of the F_2 hybrids correlated with the de-

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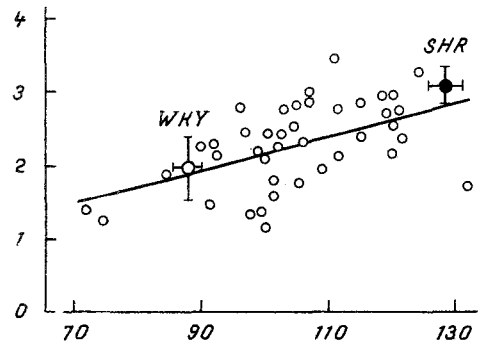

Fig. 1

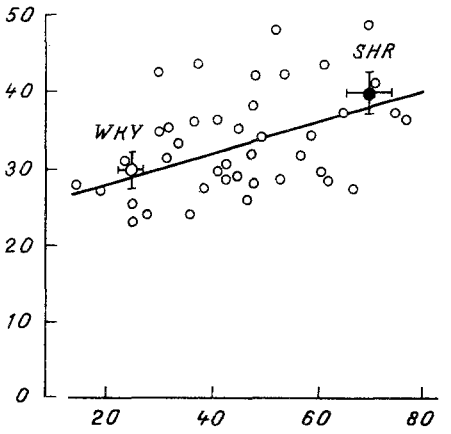

Fig. 2

Fig. 1. Correlation between BP_2 and rate of Na^+,K^+-cotransport in erythrocytes of F_2 hybrids ($r = 0.509$; $p < 0.001$). Abscissa, BP_2 (in mm Hg); ordinate, rate of Na^+, K^+-cotransport (in relative units). Here and in Fig. 2, mean values and standard error of parameters obtained for animals of original lines also are shown.

Fig. 2. Correlation between ΔBP and calcium concentration in erythrocytes incubated in the presence of orthovanadate in F_2 hybrids ($r = 0.452$; $p < 0.005$). Abscissa, ΔBP (in mm Hg); ordinate, calcium concentration in erythrocytes (in nmoles/ml of cells).

crease in BP under the influence of ether anesthesia. The increase in this component of BP in conscious SHR compared with WKY may be due to increased activity of the peripheral component of the sympathetic nervous system. Unlike the calcium concentration in the erythrocytes, the value of Na^+,K^+-cotransport correlated better with the BP component that was independent of ether anesthesia (BP_2); the coefficient of correlation of this parameter with BP_1 was 0.171, i.e., three times less than with BP_2 (Fig. 2).

This approach, based on analysis of distribution of the trait in the F_2 generation and its linkage with BP, leads to the conclusion that a relationship of cause and effect exists between the physiological differences discovered at the cellular level and the development of hypertension. In fact, in the F_2 generation, after random mixing of genes of the initial lines, different discrete groups are found on the basis of the trait, in which BP differs, it must follow from this that the trait is inherited linked with that region of the genome which controls the BP level. In the presence of a logical connection between the trait studied and the mechanisms of pressure regulation, it can be asserted that this trait is of definite importance in the development of hypertension. The principles of such an approach to the study of spontaneous hypertension were described in detail previously [6].

During analysis of a sample of 41 animals we were unable to discover any discrete groups on diagrams of distribution of values of Na^+,K^+-cotransport or calcium concentration. On the basis of this observation it can be postulated that the traits which we studied are polygenic or monogenic, but with wide environmental variability, as a result of which the distribution of their values in F_2 is continuous.

The continuous distribution of the trait does not allow the conclusion to be drawn that a relationship of cause and effect exists between changes in its value and the rise of BP. This trait may either be secondary relative to the raised pressure or it may be under the control of factors independently affecting both it and the BP level. The first hypothesis in this case is unlikely, for an increase in the rate of Na^+,K^+-cotransport [5] and in the calcium concentration has been found (data are in process of preparation for publication) in erythrocytes of SHR in the prehypertensive stage. So far as the second hypothesis is concerned, the case relative to Na^+,K^+-cotransport was investigated in detail in [4], when positive correlation was found between its value and BP in F_2 rats obtained by crossing animals of the Milan hypertensive and normotensive lines. It was shown in the same investigation that erythropoiesis in bone marrow cells transplanted into F_1 hybrids from animals of the original lines leads to the development of mature erythrocytes with a level of Na^+,K^+-cotransport corresponding to that observed in the donor line. On the basis of these experiments it can be

concluded that the level of Na^+,K^+-cotransport is an internal property of erythrocytes and is not controlled by extracellular factors, including the BP level. Thus the level of Na^+,K^+-cotransport in erythrocytes can be regarded as a marker of spontaneous hypertension, whereas disturbances in this system or in the system of intracellular factors influencing it, can be regarded as one of the mechanisms determining the development of this disease.

The phenomenon of a raised calcium concentration in erythrocytes of SHR, incubated in the presence of orthovanadate, has so far received little study. All that is known is that this parameter is even more marked in SHR in which the disease follows a malignant course (spontaneously hypertensive stroke prone rats — SHRSP), and it is unchanged in hypertensive rats of the Milan strain [3]. Nevertheless, the fact that this parameter correlates with BP, as was observed in the present investigation in (SHR × WKY)F_2 hybrids, and also the existence of differences between SHR and WKY, in the prehypertensive stage, point to a significant contribution of this component of the membrane defect to the development of chronic hypertension in these animals.

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