3. S. E. Bloom and C. Goodpasture, Hum. Genet., 34, 199 (1976).
4. D. W. Clough, L. M. Kunkel, and R. L. Davidson, Science, 216, 70 (1982).
5. D. N. Cooper, Hum. Genet., 64, 315 (1983).
6. M. Ferraro and P. Lavia, Exp. Cell Res., 145, 452 (1983).
7. M. Ferraro and P. Lavia, Chromosoma, 91, 307 (1985).
8. W. M. Howell and D. A. Black, Experientia, 86, 101 (1980).
9. P. A. Jones and S. M. Taylor, Cell, 20, 85 (1980).
10. P. A. Jones and S. M. Taylor, Nucleic Acids Res., 9, 2933 (1981).
11. J. L. Mandel and P. Chambon, Nucleic Acids Res., 7, 2081 (1979).
12. J. D. McGhee and G. D. Ginder, Nature, 280, 419 (1979).
13. T. Mohandas, R. S. Sparkes, and L. J. Shapiro, Science, 24, 393 (1981).
14. M. Schmid, D. Grunert, T. Haaf, and W. Engel, Cytogenet. Cell Genet., 36, 544 (1983).
15. L. H. T. VAn der Ploeg and R. A. Flawell, Cell, 19, 947 (1980).

c-src LOCUS DETERMINES INCREASED RATE OF Na^+,K^+-COTRANSPORT AND INCREASED CALCIUM CONTENT IN (SHR × WKY)F_2 HYBRID ERYTHROCYTES

Yu. V. Kotelevtsev, D. D. Spitkovskii, S. N. Orlov, and Yu. V. Postnov

UDC 612.111.015.31:546.41].08

KEY WORDS: c-src locus; calcium; Na^+, K^+-cotransport

Spontaneously hypertensive Okamoto-Aoki rats, (SHR, Wistar-Kyoto strain) are nowadays used as an experimental model of essential hypertension in man, for which they are the closest prototype [7]. In both forms of pathology characteristic disturbances have been found in the cation-transport function of the plasma membrane in various types of cells (the so-called membrane defect), and the key role of these disturbances in the pathogenesis of these forms of primary hypertension has been demonstrated [5]. Further development of these investigations has shown that a high proportion of these membrane disturbances can be reproduced by activating intracellular protein kinases, and it has been suggested that products of cell oncogenes may participate in the genesis of the membrane disturbances in these forms of hypertension [4, 6]. Recently, by the method of restriction analysis of DNA of SHR rats and of WKY controls to them (normotensive Wistar–Kyoto rats) interlinear polymorphism of the c-src locus was found, characterized by SHR (src^S) and WKY (src^W, HindIII: 3.4 kbp, src^S, 4.1 kbp, src^W; PstI: 4 kbp, src^S, 4.6 kbp, src^W) alleles [1].

In the investigation described below linking of c-src alleles with Na,K-cotransport and the calcium content in erythrocytes (in the presence of the Ca-ATPase inhibitor, Na_3VO_4), was studied for the first time in (SHR × WKY)F_2 hybrids. Previously work wiith such hybrids demonstrated positive correlation of the above-mentioned parameters with the blood pressure [2].

EXPERIMENTAL METHOD

Male second generation hybrids between SHR and WKY, aged 8 weeks, were used. They were obtained by crossing F_1 sibs, progenies of SHR♂ × WKY♀ and SHR♀ × WKY♂ pairs. SHR and WKY rats were obtained in 1973 from the Montreal Institute of Clinical Research, and both lines of rats were maintained under strict genetic supervision in the animal house of the laboratory.

Central Research Laboratory, Ministry of Health of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 11, pp. 608-609, November, 1989. Original article submitted March 6, 1989.

 0007-4888/89/0011-1634$12.50

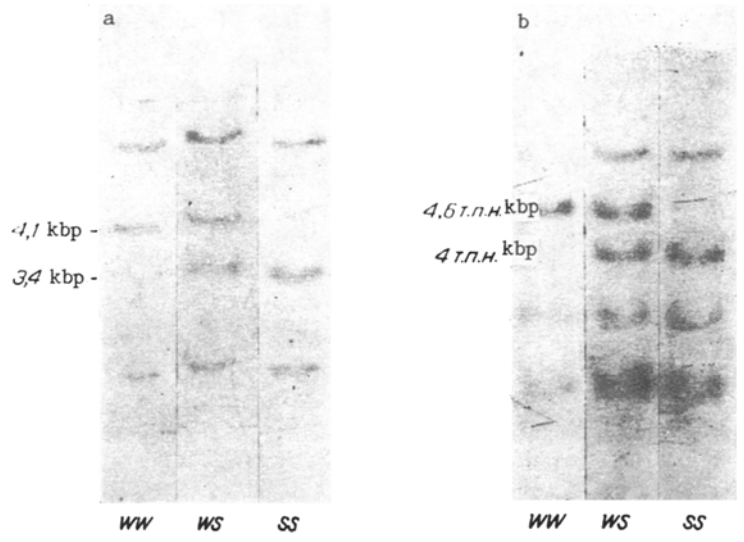

Fig. 1. Blot hybridization of DNA of (SHR × WKY)F_2 c v-src hybrids. Restriction: a) HindIII, b) PstI. WW, SS) Homozygous genotype, WS) heterozygous genotype.

TABLE 1. Physicochemical Properties of Erythrocytes of (SHR × WKY)F_2 Hybrids Differing in c-src Genotype

Serial No.	Group of of animals	n	Na,K-ATPase	Na,K-cotransport	(Ouabain + furosemide) insensitive component of ^{86}Rb entry	Ca, μmoles/liter of cells
			μmoles/liter cells/h			
1	F_2-WW	8	1631±124	251±24	1536±77	19,08±0,76
2	F_2-SS	4	1899±251	448±96	1736±107	24,14±1,80
3	F_2-WS	14	1874±101	379±22	1578±38	22,22±1,55
4	WKY	5	1647±181	298±36	1601±89	21,08±1,93
5	SHR	5	1808±195	431±50	1588±95	40,71±3,14
	$p_{1,2}$		NS	0,03	NS	0,02
	$p_{1,3}$		NS	0,002	NS	0,03
	$p_{4,5}$		NS	0,05	NS	<0,001

The procedure of taking blood, obtaining erythrocytes, and determining Na,K-ATPase activity (ouabain-inhibited component of the rate of entry of ^{86}Rb), the Na,K-cotransport (ouabain + furosemide), and the insensitive component of the rate of entry of ^{86}Rb, and the quantity of calcium accumulated by erythrocytes in the presence of orthovanadate, were all determined by methods described previously [3, 6].

Isolation of DNA from rat liver tissue, and restriction and electrophoresis of the DNA were carried out as described in [1]. DNA transfer was carried out in 0.4 M NaOH on a nylon membrane (Zeta-probe, Bio-Rad, USA). Insertion of the v-src gene was labeled by nick-translation in the presence of one radioactive nucleotide. Specific activity of the label was not less than 5×10^7 cpm, and its concentration 2×10^6 cpm/ml of hybridization buffer. Hybridization was carried out at 42°C in buffer: 50% formamide, 5 × SSC, 0.5% "Blotto" dried milk, 0.1 mg/ml yeast tRNA, and 1% SDS. The sample was washed for 2 × 15 min successively with 1 × SSC, 0.5 × SSC at 20°, 0.2 × SSC, and 0.1 × SSC at 65°C in the presence of 0.1% SDS. The duration of exposure was 10-14 days at −70°C.

EXPERIMENTAL RESULTS

On the basis of detection of restriction fragments corresponding to src^S and src^W alleles, the male hybrids were divided into three groups: src^{SS} (n = 4), src^{WS} (n = 14), and src^{WW} (n = 8). Hybridization maps corresponding to these genotypes are shown in Fig. 1. Genotypes determined on the basis of HindIII and PstI restriction fragments coincided in all cases. Table 1 gives mean values of Na,K-ATPase, Na,K-cotransport, passive entry of Rb^+, and the calcium content, calculated for groups of hybrids formed in accordance with their genotypes for the src locus. It will be clear from Table 1 that the value of Na,K-cotransport for the group of hybrids with src^{WW} genotype coincides with the corresponding values for WKY and differs substantially from src^{WW}, which has intermediate value, and src^{SS}, coinciding with the

value of SHR. These data definitely indicate that the c-src locus belongs to the part of the genome determining the magnitude of Na^+,K^+-cotransport. Analysis of the situation, when one feature is quantitative, makes isolation of crossover individuals and, correspondingly, calculation of the probability of linkage, difficult. Nevertheless, the absence of individuals with the src^{ww} genotype, and of values of Na,K-cotransport for src^{ws} falling within the confidence interval, and vice versa, is evidence of the sufficiently close linkage of c-src with the locus determining the magnitude of Na,K-cotransport. However, strict proof of their identity requires analysis of a larger number of individuals and of additional DNA markers close to c-src.

The calcium content in src^{ww} erythrocytes was the same as that in WKY, and lower than in src^{ws} and src^{ss} which, in turn, was substantially lower than in SHR. The Ca^{++} concentration is evidently controlled by several unlinked genes, and the locus inherited as linked with c-src has a significant effect on this trait.

The results are evidence that a fragment of the genome close to the c-src gene determines the magnitude of Na,K-cotransport and affects the calcium content in the erythrocytes — parameters which correlate with the blood pressure in F_2 hybrids [2].

LITERATURE CITED

1. Yu. V. Kotelevtsev, D. A. Brashishkite, D. D. Spitkovskii, and Yu. V. Postnov, Byull, Éksp. Biol. Méd., No. 5, 585 (1988).
2. Yu. V. Kotelevtsev, S. N. Orlov, N. I. Pokudin, et al., Byull. Éksp. Biol. Med., No. 4 456 (1987).
3. S. N. Orlov, N. I. Pokudin, and Yu. V. Kotelevtsev, Kardiologiya, No. 1, 57 (1988).
4. Yu. V. Postnov, Kardiologiya, No. 1,, 98 (1987).
5. Yu. V. Postnov and S. N. Orlov, Primary Hypertension as Pathology of Cell Membranes [in Russian], Moscow (1987).
6. Yu. V. Postnov, G. M. Kravtsov, S. N. Orlov, et al., Hypertension, 12, 267 (1988).
7. Y. Yamori, Hypertension, ed. by P. Slight and E. Freis, London (1982), pp. 56-77.