Partitioning of Erythrocytes from Spontaneously Hypertensive and Wistar-Kyoto Rats

HARRY WALTER, ELLIS R. LEVIN, EUGENE J. KROB, AND STEVEN D. MILLS

SUMMARY The charge-associated and non-charge-associated (probably lipid-related) surface properties of erythrocytes from spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY), from which SHR were originally derived, were studied by cell partitioning in dextran–polyethylene glycol aqueous phase systems. A major difference was found in the surface charge-associated and lipid-related properties of red blood cells from SHR and WKY: the cells from WKY had the higher partition ratio in both charge-sensitive and non-charge-sensitive phases. No difference in partitioning could be found between any two SHR nor between any two WKY. The SHR and WKY erythrocytes showed the same difference when compared with one another even when rats had the same blood pressure. When red blood cells from SHR with different blood pressures were compared, there still was no difference in their surface properties. These results suggest that the differences in both charge-associated and lipid-related surface properties of erythrocytes from SHR and WKY are strain-specific (i.e., genetic) but that there is no correlation, reflected by partitioning, between red blood cell surface properties and the degree of the rats' hypertension. (Hypertension 8:533–539, 1986)

KEY WORDS • surface charge • membrane lipid • dextran • polyethylene glycol • aqueous phase systems • erythrocyte membrane

O KAMOTO-AOKI spontaneously hypertensive rats (SHR) are a genetically inbred strain that serves as an animal model of hypertension.1,2 Compared with normotensive Wistar-Kyoto rats (WKY), from which SHR were derived, SHR display increased sympathetic activity, which may account for their increased blood pressure.3 Various other physical differences exist between SHR and WKY, and the question arises of whether these differences manifest themselves in a coordinate fashion with the expression of hypertension.

Differences in electrophoretic mobility of erythrocytes obtained from SHR and WKY were reported recently.4 In the present study, the surface properties of red blood cells from SHR and WKY were examined by a sensitive and novel method.

Membrane surface differences, both charge-associated and lipid-related, between closely related cell populations can be detected by cell partitioning in dextran–polyethylene glycol aqueous phase systems.5–7 The procedure consists of isotopically labeling (in the case of red blood cells with sodium [31Cr]chromate) aliquots of two cell populations and mixing these with an excess of unlabeled cells with which they are to be compared. Mixtures of labeled and unlabeled cells of the same type are also prepared as controls to ascertain that the labeling procedure per se does not influence the cells' partitioning behavior. These four mixtures are separately but simultaneously subjected to counter-current distribution (CCD), a multiple extraction procedure, in a charge-sensitive or a non-charge-sensitive phase system. Distribution curves are analyzed for total cells (in terms of hemoglobin absorbance) and labeled cells (in terms of counts per minute). Changes in relative specific activities through the distribution curves are indicative of subtle differences in surface properties of erythrocyte populations.
This method was established by examining the behavior of cell populations, the relative partition ratios of which were known.6 7 It was then applied to detecting surface differences between human red blood cells of different cell age, which previously had not been detectable. The power of the method is demonstrated by the finding that surface differences unrelated to ABO (or MNSs) blood group exist between erythrocytes from arbitrarily chosen (presumably hematologically normal) subjects.8 Studies on erythrocytes from inbred and outbred rats indicate that surface differences among outbred rats and between rat strains are detectable by partitioning, while no difference is evident when animals from inbred strains are examined.9 These results suggest that the differences detected by partitioning in these experiments are genetic rather than acquired.

The present study examined the partitioning behavior, in charge-sensitive and non-charge-sensitive phase systems, of erythrocytes from SHR and WKY to determine 1) whether surface differences between erythrocytes from SHR and WKY are detectable by partitioning and 2) whether such differences, if found, correlate with the degree of hypertension (either between WKY and SHR or among SHR).

Materials and Methods

Male and female SHR and WKY (Harlan Sprague-Dawley, Indianapolis, IN, USA) ranging in age from 6 to 40 weeks were used in these experiments. No sex-associated difference in the behavior of red blood cells from different animals was observed in our experiments. Both WKY and SHR were fed an identical diet of Wayne Rodent Blox (Continental Grain, Chicago, IL, USA) with a total fat content of 4.4%, of which about 37% was linoleic and another 37% oleic acid.

The rats were anesthetized with ketamine, 10 mg i.m., and polyethylene-50 tubing (Clay-Adams, Parsippany, NJ, USA) was inserted into the carotid artery after careful neck dissection according to the method of Popovic and Popovic.10 The distal tip was left in the aortic arch, and the catheter was sutured in place and exteriorized to the nape of the neck. It was held in place at the neck with a protective metal button and spring (Harvard Bioscience, Cambridge, MA, USA). The rats were permitted to recover for 2 days before the experiments began. Blood pressure was recorded directly with the catheter attached to a 23Db Gould transducer (Oxnard, CA, USA) and Beckman 511A polygraph (Fullerton, CA, USA) while the rats were freely moving in a 45 × 22 × 22-cm plastic cage. Recordings were made at 10-minute intervals to establish basal mean arterial pressure, defined as four consecutive readings within 5 mm Hg of each other.

Blood Collection

Rats were bled by heart puncture, and 10 ml of blood was collected in 4 ml of acid-citrate-dextrose anticoagulant. Red blood cells were used in the experiments within 1 week of collection.

Labeling and Preparation of Erythrocytes

Isotopic labeling of red blood cells with $^{51}$Cr has been described in detail, as have the counting procedures used.5 7 Approximately 10 μCi of $^{51}$Cr was used per milliliter of an aliquot of the anticoagulated blood suspension just described (giving rise to a specific activity of approximately 2500 cpm per hemoglobin absorbance at 540 nm on our scintillation well counter; see Concurrent Distribution). Both labeled and an aliquot of unlabeled cells were then washed five times with phosphate buffered saline, pH 7.0.

The preparation of mixtures of labeled and unlabeled erythrocytes for analysis by CCD has been described previously in detail.5 8 In brief, to compare the surface properties of erythrocytes from two rats, A and B (i.e., 2 SHR, 2 WKY, or 1 of each strain), 0.15 ml of $^{51}$Cr-labeled red blood cells (washed as indicated) is pipetted into a centrifuge tube containing 5 ml of phosphate buffered saline. Then, 0.6 ml of unlabeled, washed red blood cells with which the labeled red blood cells are to be compared is pipetted into the same tube. Cells in the tubes are gently mixed and centrifuged. The supernatant solution is discarded, and the packed cells are used to make the “load mix” for CCD (see Concurrent Distribution). An excess of unlabeled cells is mixed with labeled cells so that subsequent analysis of cell distribution curves by hemoglobin absorbance will reflect primarily the unlabeled cell population, while the distribution of the labeled cell population can be obtained by isotope counting. Four mixtures are examined in each experiment: $^{51}$Cr-labeled red blood cells A + unlabeled red blood cells B (A + B); $^{51}$Cr-labeled red blood cells B + unlabeled red blood cells A (B + A); $^{51}$Cr-labeled red blood cells A + unlabeled red blood cells A (A + A); $^{51}$Cr-labeled red blood cells B + unlabeled red blood cells B (B + B). Mixing $^{51}$Cr-labeled red blood cells with unlabeled red blood cells that are of the same population is an essential control to indicate that $^{51}$Cr-labeling procedures per se have no effect on the surface properties.5 8

Phase Systems

The preparation of two-polymer aqueous phase systems and their physical properties have been described previously.3 Two phase systems were employed in the present study. System 1 contained 5% (wt/wt) dextran T500 (lot G1 21917; Pharmacia Fine Chemicals, Piscataway, NJ, USA), 4% (wt/wt) polyethylene glycol 8000 (Carbowax 8000, formerly 6000, Union Carbide, New York, NY, USA), 0.09 M sodium phosphate buffer, pH 6.8, and 0.03 M NaCl. System 2 contained 5% (wt/wt) dextran, 3.5% (wt/wt) polyethylene glycol, 0.15 M NaCl, and 0.01 M sodium phosphate buffer, pH 6.8. System 1 has an electrostatic potential difference between the phases (approximately 2-3 mV)11 and is charge-sensitive, while System 2 does not have a potential difference and is non-charge-sensitive (see Reference 5 for detailed discussion).

After preparation, phase systems were equilibrated in a separatory funnel at 4 to 5°C. The polyethylene
glycol-rich top and the dextran-rich bottom phases then were separated.

Countercurrent Distribution

For CCD, 0.25 ml of each of the labeled and unlabeled red blood cell mixtures (i.e., A + B, B + A, A + A, B + B) was suspended in 2.75 ml of top phase of the system to be used (load mix). The CCD was performed in an automated apparatus built by the Workshop, Chemical Center, University of Lund, Sweden. It is constructed of two Plexiglas plates with 120 concentric cavities. The bottom (or stator) plate cavities have a capacity of 0.7 ml; there is a hole above each cavity in the top (or rotor) plate for loading of cells and phase. All cavities are loaded with 0.5 ml of bottom phase. Each of three adjacent cavities at cavities 0 to 2, 30 to 32, 60 to 62, and 90 to 92 receives 0.7 ml of one of the load mixes (A + A, B + B, A + B, or B + A). All other cavities (i.e., 3–29, 33–59, 63–89, 93–119) receive 0.7 ml of top phase. The plates on the automatic unit are then shaken in a rotary manner (at about 180 rpm with a 15-mm displacement) for 22 seconds, which mixes cells and top and bottom phases in the load cavities (i.e., those containing cells). The shaking stops, and the phases are permitted to settle (separate) for a preset length of time. Some cells prefer the top phase and some the interface depending on the physical properties of the phases and the surface properties of the cells. After the phases settle, the top plate rotates through 3 degrees while the bottom plate remains stationary. Thus, the top phase in each cavity is carried forward to the next cavity in the bottom plate. Those cells that were suspended in the top phase are carried to the next cavity, where they are reextracted with fresh bottom phase. Those cells that were at the interface and remained behind in the bottom plate cavity are extracted with fresh top phase. In this manner, the cycle is repeated (30 times in the present study): mixing, settling, transfer, and so on.

At the end of a CCD run, the cavities are emptied, by means of a collection ring, directly into centrifuge tubes and the cells are obtained from each tube by centrifugation. Total cell recovery is generally between 70 and 85%. The cells are lysed, and the total cell distribution is obtained by measuring the hemoglobin absorbance at 540 nm. The distribution of labeled cells is obtained by isotope counting in a Beckman scintillation well counter as previously described.

Presentation of Data

Total cell distribution in terms of hemoglobin absorbance indicates primarily the unlabeled cell population in each mixture, since these are the cells present in great excess. The labeled cell population is shown in counts per minute. A relative specific activity is also given through the distribution curves and reflects the extent of displacement and, hence, of difference between any two such red blood cell populations. It is defined as (cpm/unit hemoglobin absorbance in a given cavity)/(cpm/unit hemoglobin absorbance in the original mixed cell population before CCD).

Results

Background

When mixed above certain concentrations, aqueous solutions of dextran and of polyethylene glycol give rise to immiscible, liquid two-phase systems with a polyethylene glycol-rich top phase and a dextran-rich bottom phase. Such systems, being aqueous, can be buffered and rendered isotonic. They have proved to be useful for the separation and subfractionation of cell populations on the basis of surface properties by partitioning. Depending on the polymer concentrations and the ionic composition and concentration, the phase systems have greatly differing physical properties. These physical properties interact with the surface properties of cells to give cell partitions based predominantly on charge-associated, non-charge-related (i.e., probably lipid-related), or receptor-dependent parameters. For example, although both dextran and polyethylene glycol are nonionic polymers, some salts (e.g., phosphates) have different affinities for the two phases. A Donnan potential results between the phases (top phase positive). The cell partition ratio (i.e., quantity of cells in the top phase as a percentage of total cells added) will, in such phases, be charge-associated. Other salts (e.g., NaCl) have equal affinities for the two phases, and such systems have no potential difference between the phases. At lower polymer concentrations cell partitioning in such systems depends on non-charge-associated properties. An excellent correlation has been found, in non-charge-sensitive systems, between the partition ratio of red blood cells from different species and the cells’ membrane ratio of polyunsaturated to monounsaturated fatty acids, a lipid parameter.

Comparison of Surface Properties of Erythrocytes from SHR and WKY

We compared the surface properties of erythrocytes from WKY (“A”) and SHR (“B”) using the mixed, labeled cell procedure outlined in Materials and Methods. Figures 1 and 2 show typical results obtained in charge-sensitive and non-charge-sensitive phase systems, respectively. When labeled cells were mixed with unlabeled cells from the same rat (A + A, B + B) there was perfect overlap of labeled and total cells. This finding shows, as has previously been pointed out, that the labeling of red blood cells with 51Cr does not, per se, affect the cells’ partitioning behavior. Both phase systems showed differences in surface properties of red blood cells from SHR and WKY (see Figures 1 and 2). This is indicated by the displacement of total cell distribution curves from isotope curves in A + B and B + A. Note that in A + B in both figures labeled red blood cells A had a higher partition ratio (i.e., to the right) than did red blood cells B. When labeled red blood cells B were mixed with unlabeled red blood cells A (B + A), red blood cells A again had a higher partition ratio than did red blood cells B. This finding indicates that we are not dealing with an artifact but with a real difference between surface proper-
FIGURE 1. Typical countercurrent distribution (CCD) patterns of mixtures of isotopically labeled and unlabeled erythrocytes from one WKY (A) and one SHR (B). Aliquots of erythrocytes from each rat were labeled with $^{51}$Cr-chromate. Labeled cells were mixed with an excess of unlabeled, washed red blood cells from the other rat ($A + B$, $B + A$). As control, aliquots of labeled cells were also mixed with an excess of unlabeled cells from the same rat ($A + A$, $B + B$). The four mixtures were subjected to CCD in a dextran-polyethylene glycol aqueous phase system that reflects charge-associated surface properties. Phase system composition: 5% (wt/wt) dextran T500, 4% (wt/wt) polyethylene glycol 8000, 0.09 M sodium phosphate buffer (pH 6.8), and 0.03 M NaCl. Thirty transfers were completed at 4 to 5 °C using a settling time of 6 minutes and a shaking time of 22 seconds. Closed circles indicate total cell distribution in terms of hemoglobin absorbance at 540 nm; open circles show the distribution of labeled cells (in cpm); triangles show the relative specific activities (with 1.0 being the specific activity of the original unfractionated cell mixture in each case). Although results are typical of those obtained with erythrocytes from randomly selected WKY and SHR, the rats used in these experiments were selected under a constraint: both had the same blood pressure. (For additional details see text and References 1—4.)

FIGURE 2. Experiment as in Figure 1 except that the phase system used measured non-charge-related (probably lipid-related) parameters. Phase system composition: 5% (wt/wt) dextran, 3.5% (wt/wt) polyethylene glycol, 0.15 M NaCl, and 0.01 M sodium phosphate buffer (pH 6.8). Settling time used was 7 minutes. Although results are typical of those obtained with erythrocytes from randomly selected WKY and SHR, the rats used in these experiments were selected under a constraint: both had the same blood pressure. Symbols are explained in Figure 1.
ties of erythrocytes from A and B and that A had a higher and B a lower partition ratio.8

The results shown in Figures 1 and 2 are typical of those obtained in three separate experiments with erythrocytes from randomly selected SHR and WKY over a wide age range. The data depicted in these particular figures were, however, obtained with 6- to 7-week-old SHR and WKY selected with a constraint: they had the same blood pressure (mean arterial pressure, 97 mm Hg).

Analogous experiments to those just described were performed with red blood cells from WKY (data not shown). No difference was found between the erythrocytes of any two WKY (i.e., there was perfect overlap of labeled and unlabeled red blood cells).

Experiments, again analogous to those just described, were undertaken with SHR. Figures 3 and 4 show typical results obtained in charge-sensitive and non-charge-sensitive phases, respectively, when erythrocytes from two SHR were compared. Further-

**Figure 3.** Experiment as in Figure 1 except that erythrocytes from two SHR were used. These results can be obtained with any two randomly selected SHR. The rats used in these experiments were selected under a constraint: they had appreciably different blood pressures.

**Figure 4.** Experiment as in Figure 2 except that erythrocytes from two SHR were used. These results can be obtained with any two randomly selected SHR. The rats used in these experiments were selected under a constraint: they had appreciably different blood pressures.
more, the data depicted in these figures were obtained with red blood cells from two SHR with differing blood pressures. By examining A + B and B + A in Figures 3 and 4, one can clearly see that no difference in red blood cell surfaces, as reflected by partitioning, was evident, even when SHR with different blood pressures (mean arterial pressures ranging from 144 to 179 mm Hg) were examined.

Discussion

Partitioning of cells in dextran–polyethylene glycol aqueous phase systems is a highly sensitive and versatile method of studying cell surfaces. The sensitivity stems from the fact that the parameters determining the partition ratio are related exponentially to it. For example, membrane charge would be reflected more sensitively by cell partitioning than by cell electrophoresis, in which cell charge and mobility are related linearly. The versatility is due to the fact that appropriate selection of phase system composition permits one to obtain information on charge-associated or non-charge-related surface properties.

In the current study, the relative surface properties of erythrocytes from SHR and WKY were examined by partitioning to determine their relationship to hypertension. By CCD of mixtures of labeled erythrocytes from SHR and WKY and unlabeled erythrocytes from WKY and SHR, respectively, in charge-sensitive (see Figure 1) and non-charge-sensitive (see Figure 2) phases, we have found strain-specific red blood cell surface differences. Red blood cells from SHR had a lower partition ratio than did erythrocytes from WKY in charge-sensitive and non-charge-sensitive phases. This finding is indicative of a lower membrane charge and perhaps, by analogy with results obtained with red blood cells from different species, a lower ratio of membrane polyunsaturated to monounsaturated fatty acids in red blood cells from SHR than in those from WKY. Differences in electrophoretic mobilities between erythrocytes from the two rat strains have been reported: those from SHR had the lower mobility than did erythrocytes from WKY.

Surface differences of erythrocytes between different rat strains (e.g., Lewis and Sprague-Dawley) have been reported previously. Thus, although SHR were initially derived from WKY, surface differences between these strains’ red blood cells are not necessarily surprising. Since the results in Figures 1 and 2 were obtained with blood pressure–matched 6- to 7-week-old rats, one can conclude that blood pressure per se is not related directly to the described differences in the red blood cells’ surface properties.

Experiments comparing red blood cells between WKY revealed no differences detectable by partitioning; nor were differences evident between erythrocytes from different SHR (see Figures 3 and 4). These results could be considered analogous to those obtained with erythrocytes from different rats belonging to the highly inbred Lewis strain. One should note, however, that erythrocytes from different SHR with appreciably different blood pressures also had no detectable surface differences (see Figures 3 and 4). These results indicate that the differences in surface properties between SHR and WKY are strain-specific and genetic but are not related directly to the degree of existing hypertension.

Important differences exist between SHR and WKY in addition to the expression of hypertension. The exact inheritance of such differences or possible correlations with the hypertension-producing genetic locus are unknown. Indications are that three or four genes, including at least one major non-sex-linked gene, code for hypertension in the SHR. Although the gene locus controlling vascular smooth muscle response in SHR has been determined, little is known about the genetic predisposition that results in differences in erythrocyte electrophoretic mobilities between SHR and WKY.

Our results indicate that there is no correlation between the degree of hypertension and the charge-associated or lipid-related surface properties of erythrocytes from SHR and WKY. Similarly, the hyperactivity of the SHR has been found to be independent of blood pressure. Although we cannot exclude a common genetic locus for the onset of several physical traits in the SHR, the degree of expression of the traits measured in the present study apparently is not linked to the degree of hypertension.

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