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Effect of osmolality on erythrocyte rheology and perfusion of an artificial microvascular network

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ABSTRACT

Plasma sodium concentration is normally held within a narrow range. It may however vary greatly under pathophysiological conditions. Changes in osmolality lead to either swelling or shrinkage of red blood cells (RBCs). Here we investigated the influence of suspension osmolality on biophysical properties of RBCs and their ability to perfuse an artificial microvascular network (AMVN). Blood was drawn from healthy volunteers. RBC deformability was measured by osmotic gradient ektacytometry over a continuous range of osmolalities. Packed RBCs were suspended in NaCl solutions (0.45, 0.6, 0.9, 1.2, and 1.5 g/dL), resulting in supernatant osmolalities of 179 ± 4, 213 ± 1, 283 ± 2, 354 ± 3, and 423 ± 5 mOsm/kg H_2O. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were determined using centrifuged microhematocrit. RBC suspensions at constant cell numbers were used to measure viscosity at shear rates ranging from 0.11 to 69.5 s^{-1} and the perfusion rate of the AMVN. MCV was inversely and MCHC directly proportional to osmolality. RBC deformability was maximized at isosmotic conditions (290 mOsm/kg H_2O) and markedly decreased by either hypo- or hyperosmolality. The optimum osmolality for RBC suspension viscosity was shifted toward hyperosmolality, while lower osmolalities increased suspension viscosity exponentially. However, the AMVN perfusion rate was maximized at 290 mOsm/kg H_2O and changed by less than 10% over a wide range of osmolalities. These findings contribute to the basic understanding of blood flow in health and disease and may have significant implications for the management of osmotic homeostasis in clinical practice.

Introduction

Plasma osmolality is primarily determined by the plasma sodium concentration. It is held within narrow limits under normal physiological conditions. Alterations in plasma osmolality are readily sensed by hypothalamic receptors in the brain, which initiates compensatory mechanisms such as water intake in the case of hyperosmolality or water excretion in the case of hypo-osmolality. In hypo-osmolality, the secretion of antidiuretic hormone ADH is suppressed, which leads to decreased water reabsorption in the collecting tubules of the kidney and thereby increases water excretion. In hyperosmolality, the body reacts with thirst-driven water ingestion and an increased ADH secretion leading to water retention in the kidney (Reynolds et al., 2006; Danziger and Zeidel, 2014).

This normal homeostasis of plasma sodium concentration and osmolality can be disturbed under pathophysiological conditions and represents the most common clinical electrolyte disorder (Reynolds et al., 2006), which is associated with a considerable morbidity and mortality when severe (Arieff, 1986; Kumar and Berl, 1998). However, even mild hypo-osmolality caused by hyponatremia is associated with a poor outcome in chronic heart failure (Klein et al., 2005). Hyponatremic hypo-osmolality of severe degree can occur in the syndrome of inappropriate ADH secretion (SIADH), e.g., after brain injury, or when an excess of free water is either ingested or infused during medical treatment. Symptoms are nausea, malaise and headache, followed by lethargy, disorientation, seizure, coma, and even death in severe hyponatremia (Ellis, 1995). On the other hand, hypernatriemic hyperosmolality, which is much less common, is seen in diabetes insipidus due to either a lack of ADH or an unresponsiveness of the kidney to ADH. Symptoms of hypernatriemic hyperosmolality are similar to symptoms of hyponatremic hypo-osmolality: initial unspecific symptoms such as anorexia, restlessness, nausea, and vomiting are followed by more severe neurological symptoms such as lethargy, stupor, or coma (Reynolds et al., 2006; Shah et al., 2014).

The neurological symptoms are caused by an osmotic gradient, which is generated between the extracellular space and the intracellular compartment of brain cells. In case of hypo-osmolality, water moves into neuronal cells and causes cell swelling and tissue edema (McManus et al., 1995; Manley et al., 2000); in hyperosmolality, water is lost from the cells which leads to cell shrinkage. Both conditions

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impair cell function. Cell swelling and shrinkage is induced in every cell exposed to osmotic gradients, including blood cells (Gallagher, 2013). In hypo-osmolality, the red blood cells (RBCs) increase their volume and thereby sphericity and at the same time reduce the cellular viscosity determined by the intracellular hemoglobin concentration (Reinhart and Chien, 1985). On the other hand, hyperosmolality leads to a volume reduction but increased cellular viscosity (Reinhart and Chien, 1985). These opposing changes could have an influence on RBC deformability and thus on microvascular blood flow. It was the aim of the study to investigate the role of osmolality of the suspending medium on RBC deformability, RBC suspension viscosity, and the ability of RBCs to perfuse an artificial microvascular network.

Materials and methods

Sample preparation

Solutions with different osmolalities were prepared as follows. Saline (0.9% NaCl, 277 mOsm/kg H_2O) was used as a basis. For hypo-osmolar solutions, it was diluted 1:1 and 2:1 with distilled water, which resulted in measured osmolalities of 136 and 182 mOsm/kg H_2O, respectively. Higher osmolalities were obtained by adding either 30 mg or 60 mg sodium chloride (Sigma-Aldrich, St. Louis, MO, USA) to 10 mL of saline, yielding osmolalities of 373 and 466 mOsmol/kg, respectively. Osmolalities were measured with a vapor pressure osmometer (Vapro 5520, Wescor Inc., South Logan, Utah, USA). All experiments described below were completed within 2 h.

Blood was taken by venipuncture from healthy volunteers (age range 20–65 years), who gave their informed consent to the *in vitro* study. Tubes containing 1.8 mg/mL K_2EDTA as an anticoagulant were used. Routine hematological analyzers (Sysmex XT-1800i, Sysmex Digitana Co, Horgen, Switzerland; Medonic M-series, Boule Medical AB, Stockholm, Sweden in the USA) were used to determine RBC count and mean cellular hemoglobin (MCH). Hematocrit (Hct) was determined by microcentrifugation. For each sample, 3 uncoated hematocrit glass tubes (length 75 mm) were filled and centrifuged for 5 min in a micro-hematocrit centrifuge (IEC MB Centrifuge, Damon), the Hct was determined (Hawksley Micro-hematocrit Reader, Lancing, Sussex, UK), and the mean value was calculated. The Hct was used to calculate the mean cellular volume in the actual suspension with a given osmolality (MCV = Hct × 10/RBC count). This MCV was then used to calculate the MCHC (MCHC = MCH/MCV). Samples of RBCs incubated at high and low osmolality were fixed in 1% glutaraldehyde and prepared for scanning electron microscopy as described previously (Reinhart et al., 2014).

Ektacytometry

RBC deformability was analyzed by laser diffraction in an ektacytometer (Technicon, Bayer, Leverkusen, Germany) using an osmoscan mode (Deuel et al., 2012). Blood from 6 healthy volunteers (age range 28–50 years) anticoagulated with EDTA as described above was used. Aliquots of 500 μL whole blood were mixed with 3 mL of an isotonic 20% dextran 70 kDa solution and inserted into the ektacytometer. RBC elongation as a measure of deformability was then registered continuously while the osmotic conditions were gradually changed, going from hypo-osmolality to hyperosmolality. The osmotic gradient was generated by adding sodium chloride to the solution in one compartment of the gradient mixer. The osmolality of the suspension was measured by determining the conductivity of the solution close to the diffractometer of the instrument, which had been calibrated by a series of different osmolalities by cryoscopic osmometry (Osmomat 030, Gonotec GmbH, Berlin, Germany). RBC deformability was plotted against the suspension osmolality. The analog output was digitized using a 12-bit A/D-converter (NI USB-6008, National Instruments, Austin, TX, USA). The deformability index (DI) was calculated at given osmolalities used in the other experiments (179, 213, 283, 354, and 420 mOsm/kg H_2O).

Viscometry

EDTA-blood from 8 healthy volunteers was centrifuged at 1500 × *g* for 5 min. The plasma and buffy coat were discarded. Volumes of 500 μL packed RBCs were added to 750 μL of NaCl solutions with increasing osmolalities (see above). The RBC count and hematocrit of these samples were measured. The hematocrit was then adjusted to 40% in the isotonic aliquot (283 mOsm/kg H_2O) by removing a calculated volume of suspending medium. The resulting actual RBC count in the 283 mOsmolar (isosmotic) sample was then used to adjust the same RBC count in the other samples. The final centrifuged hematocrit values of the different osmolality samples were calculated accordingly. RBC suspension viscosities were measured with a Couette viscometer (Contraves LS 30, ProRheo, Althengstett, Germany) at room temperature (20–22 °C) at shear rates of 69.5, 27.7, 11.0, 3.23, 0.95, 0.28, and 0.11 s^{-1}.

Perfusion of artificial microvascular networks

Artificial microvascular network (AMVN) devices were fabricated using previously described methods (Burns et al., 2012; Sosa et al., 2014; Forouzan et al., 2012; Shevkoplyas et al., 2006). Each polydimethylsiloxane (PDMS) AMVN device consisted of three identical networks of microchannels, each with a separate domed inlet (4 mm diameter), that all converged into a common domed outlet (1.5 mm diameter) connected to an adjustable water column (Burns et al., 2012; Sosa et al., 2014). The interconnected microchannels making up the AMVN had a depth of 5 μm and ranged in width from 5 to 70 μm. The layout of the AMVN was inspired by the microcapillary architecture of rat mesentery and has been described in detail previously (Burns et al., 2012).

Fresh blood antricoagulated with EDTA from 5 healthy volunteers was centrifuged at 1500×*g* for 5 min. One hundred microliters of packed RBCs was then resuspended in 150 μL NaCl solutions (target hematocrit 40% at isotonicity) of increasing osmolalities (see above). To perform the measurement, 60 μL samples of resuspended RBCs were loaded into the inlets of the AMVN device with a conventional 10–100 μL pipette tip; image acquisition was initiated and the driving pressure was increased to 20 cmH_2O. Image acquisition continued for 10 min. An inverted bright-field microscope (IX71, Olympus America Inc., Center Valley, PA, USA) equipped with a high-speed camera (MC1362, Mikrotron GmbH, Unterschleissheim, Germany) was used to acquire images. The camera was programmed to acquire sets of 10 frames at 100 fps every 10 s. A custom algorithm in MATLAB (The Math Works Inc., Natick, MA, USA) computed the mean cell velocity in the microchannel exiting each network unit from the average change in cell position between consecutive timed images for each image set. The AMVN perfusion rate was then calculated as the product of the mean cell velocity and the cross-sectional area of the exit microchannel (350 μm^2) (Burns et al., 2012).

Results

The initial osmolalities of the suspending solutions were 138, 183, 277, 376, and 468 mOsm/kg H_2O. The suspension of RBCs in these solutions with a target hematocrit of 40% led to an osmotic equilibration between the suspending solution and the cytoplasm of RBCs. After centrifugation, the osmolalities in the supernatant were 179 ± 4, 213 ± 1, 283 ± 2, 354 ± 3, and 423 ± 5 mOsm/kg H_2O, respectively. Hypo-osmolality induced water uptake by the discocytes leading to spherical swelling with a reduction of the cell diameter (Fig. 1a). On the other hand, hyperosmolar conditions dehydrated RBCs, which became more flattened discocytes (Fig. 1b). The difference in diameter seen in Fig. 1 may be exaggerated due to the isotonic glutaraldehyde fixing solution, which might have decreased osmotically the existing RBC