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SHORT COMMUNICATION

Erythrocyte Ion Transport in Rats Subjected to Acute and Chronic Hypobaric Hypoxia

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Summary
Our study addresses selected parameters of rat erythrocyte ion transport (Na^+-K^+ pump, Na^+-K^+-$2Cl^-$ cotransport, and passive cation fluxes) after acute or chronic hypoxia exposure. We did not find any significant change of ion transport after acute hypoxia. However, chronic hypoxia could modify ion transport because the affinity of Na^+-K^+ pump for intracellular Na^+ seems to be decreased.

Key words
Erythrocyte • Intracellular Na^+ content • Na^+ transport • Rb^+ (K^+) transport • Acute and chronic hypobaric hypoxia

Erythrocytes represent a good model system for the study of membrane transport because of their simplicity, easy procurement and relative homogeneity (Gibson *et al.* 2000). The high content of polyunsaturated fatty acids in their membrane and high cellular concentration of oxygen and hemoglobin make erythrocytes susceptible to oxidative damage. Under normoxic conditions, reactive oxygen species (constantly generated in erythrocytes) are mostly neutralized by their inner antioxidant enzymatic and non-enzymatic defenses such as superoxide dismutase, glutathione peroxidase, catalase or reduced glutathione (Kurata *et al.* 1993). However, under the conditions of hypoxia, autooxidation of hemoglobin is facilitated and an increased flux of superoxide radicals occurs (Rifkind *et al.* 1991). González *et al.* (2002) reported that the hypobaric hypoxia exposure (with followed reoxygenation) affects the major integral membrane protein of erythrocytes, band 3 protein, which constitutes 25 % of the total membrane protein and is a sensor for metabolically active tissues facilitating the oxygen translocation from hemoglobin to tissues.

The aim of our study was to examine the changes of Na^+ and K^+ transport in rat erythrocytes after acute or chronic hypobaric hypoxia exposure.

Our experiments were performed on Wistar rats which were housed under standard laboratory conditions (temperature 23±1 °C, 12-h light-dark cycle), were fed a standard pelleted diet and were drinking tap water *ad libitum*. One group of animals was submitted to acute hypobaric hypoxia (21-day-old rats exposed to 30 min on hypoxia simulating an altitude of 9000 m) and another group to chronic hypobaric hypoxia (5-month-old-rats exposed to intermittent hypoxia simulating an altitude of

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Table 1. Parameters of erythrocyte Na^+ and Rb^+ (K^+) transports after acute hypobaric hypoxia in 21-day-old rats

Na^+ transport (mmol/l erythrocytes/h)			
Group	**Pump**	**Cotransport**	**Leak**
Control (n=5)	8.515±0.367	2.493±0.358	9.480±0.488
Hypoxia (n=5)	7.920±0.359	2.424±0.254	9.047±0.640
Rb^+ (K^+) transport (mmol/l erythrocytes/h)			
Group	**Pump**	**Cotransport**	**Leak**
Control (n=5)	10.669±0.640	0.707±0.052	0.815±0.057
Hypoxia (n=5)	9.992±0.677	0.456±0.078*	0.823±0.061

∗ significantly different from control ($p<0.05$)

Table 2. Parameters of erythrocyte Na^+ and Rb^+ (K^+) transports after chronic hypobaric hypoxia in adult rats (aged 5 months)

Na^+ transport (mmol/l erythrocytes/h)			
Group	**Pump**	**Cotransport**	**Leak**
Control (n=6)	6.191±0.357	1.825±0.365	4.596±0.339
Hypoxia (n=7)	5.570±0.249	1.857±0.308	4.589±0.150
Rb^+ (K^+) transport (mmol/l erytrocytes/h)			
Group	**Pump**	**Cotransport**	**Leak**
Control (n=6)	5.455±0.244	1.561±0.171	0.487±0.012
Hypoxia (n=7)	5.028±0.288	1.598±0.221	0.442±0.018

5000 m for 8 h/day, 5 days a week with a total of 30 exposures). Erythrocytes were isolated from heparinized abdominal aorta blood. Blood samples were centrifuged and the buffy coat (platelets and white blood cells) was removed. One sample represents the erythrocytes from one adult rat or from 4-6 young rats (21-day-old). Cation transport mediated by the Na^+-K^+ pump, the Na^+-K^+-$2Cl^-$ cotransport system or cation leaks (movements reflecting passive membrane permeability) were studied as described earlier (Kuneš *et al.* 1994, Zicha *et al.* 1997, Vokurková *et al.* 2003). The results were expressed as mean ± S.E.M. The statistical differences were evaluated by one-way analysis of variance followed by the least significant difference test.

Table 1 shows a comparison of normoxic and acutely hypoxic immature rats (aged 21 days) which were characterized by higher transport rates compared to adult animals. There were no significant differences in Na^+ and Rb^+ (K^+) transports, except Rb^+ (K^+) cotransport which was decreased ($p<0.05$) under hypoxic conditions. This might be related to the occurrence of immature erythrocytes in hypoxic rats. The estimated values of intracellular Na^+ content were similar for controls and hypoxic rats (3.478±0.119 *vs.* 3.583±0.136 mmol/l erythrocytes). As far as erythrocytes from adult animals in chronic hypobaric hypoxia are concerned, we found significantly higher values of intracellular Na^+ content in comparison with the controls (5.981±0.180 *vs.* 4.952±0.255 mmol/l erythrocytes; $p<0.01$), but there were no significant differences in activities of the Na^+-K^+ pump, cotransport and monovalent cation leaks (Table 2). Although we did not see any significant differences in the activity of the Na^+-K^+ pump, it appears from the relationship between pump activity and intracellular Na^+ content that the affinity for intracellular Na^+ content was decreased in rats submitted to chronic hypoxia (Fig. 1). In agreement with our results, hypoxia-induced inhibition of the Na^+-K^+ pump was abolished in Na^+-loaded erythrocytes revealing no effect of O_2 on the maximal operation rate of the pump (Bogdanova *et al.* 2003)

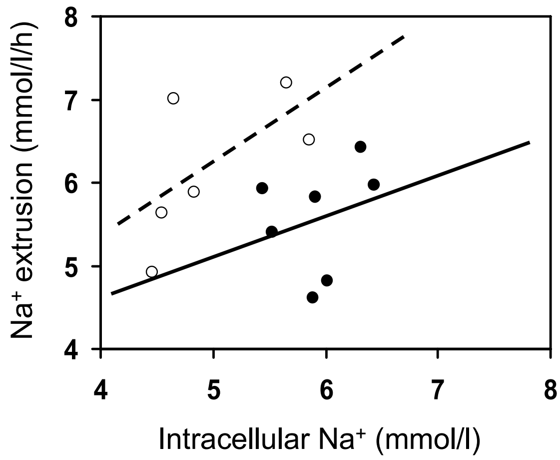

Fig. 1. The relationship between Na^+-K^+ pump activity and intracellular Na^+ content in erythrocytes from controls (open circles) and rats submitted to chronic hypoxia (full circles)

Variations in O_2 tension represent a specific signal capable of regulating the activity of many erythrocyte membrane transport proteins (Drew *et al.* 2004). In addition, the interaction between O_2 and other stimuli can be a critical determinant of erythrocyte transporter activity (Gibson *et al.* 2000). The response to O_2 is specific and selective across species and different transporters. For example, isolated mouse erythrocytes submitted to normoxic or hypoxic conditions did not change the activity of the Na^+-K^+-$2Cl^-$ cotransport but the

Na^+-K^+ pump responded to hypoxic treatment by reversible inhibition (Bogdanova *et al.* 2003). On the other hand, Drew *et al.* (2004) did not see any change in the Na^+-K^+ pump activity, but the Na^+-K^+-$2Cl^-$ cotransport was stimulated by deoxygenation in both chicken (nucleated) and human erythrocytes. To our knowledge, there are no studies evaluating erythrocyte ion transport changes induced by *in vivo* exposure of animals to acute or chronic hypoxia. It can be presumed that ion transport changes can be found in immature erythrocytes (reticulocytes) released into the circulation of animals after several days of high altitude exposure (Furukawa *et al.* 1981).

In conclusions, our results indicate that chronic but not acute hypobaric hypoxia modified ion transport in rat erythrocytes.

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References

BOGDANOVA AY, OGUNSHOLA OO, BAUER C, GASSMANN M: Pivotal role of reduced glutathione in oxygen-induced regulation of the Na^+/K^+ pump in mouse erythrocyte membrane. *J Membrane Biol* **195**: 33-42, 2003.

DREW C, BALL V, ROBINSON H, ELLORY JC, GIBSON JS: Oxygen sensitivity of red cell membrane transporters revisited. *Bioelectrochemistry* **62**: 153-158, 2004.

FURUKAWA H, BILEZIKIAN JP, LOEB JN: Potassium fluxes in the rat reticulocyte. Ouabain sensitivity and changes during maturation. *Biochem Biophys Acta* **649**: 625-632, 1981.

GIBSON JS, COSSINS AR, ELLORY JC: Oxygen-sensitive membrane transporters in vertebrate red cells. *J Exp Biol* **203**: 1395-1407, 2000.

GONZÁLEZ G CELEDÓN G, SANDOVAL M, GONZÁLEZ GE, FERRER V, ASTETE R, BEHN C: Hypobaric hypoxia-reoxygenation diminishes band 3 protein functions in human erythrocytes. *Pflügers Arch* **445**: 337-341, 2002.

KUNEŠ J, BIN TALIB HK, DOBEŠOVÁ Z, VRÁNA A, ZICHA J: Erythrocyte ion transport alterations in hypertriglyceridaemic rats. *Clin Sci* **86**: 11-13, 1994.

KURATA M, SUZUKI M, AGAR NS: Antioxidant systems and erythrocyte life-span in mammals. *Comp Biochem Physiol B* **106**: 477-487, 1993.

RAUCHOVÁ H, VOKURKOVÁ M, DOBEŠOVÁ Z, KUNEŠ J, ZICHA J: Erythrocyte ion transport in rats subjected to chronic and acute hypobaric hypoxia. *Physiol Res* **54**: 40P, 2005.

RIFKIND JM, ZHANG L, LEVY A, MANOHARAN PT: The hypoxic stress on erythrocytes associated with superoxide formation. *Free Radic Res Commun* **12-13**: 645-652, 1991.

VOKURKOVÁ M, DOBEŠOVÁ Z, KUNEŠ J, ZICHA J: Membrane ion transport in erythrocytes of salt hypertensive Dahl rats and their F2 hybrids: the importance of cholesterol. *Hypertens Res* **26**: 397-404, 2003.

ZICHA J, DOBEŠOVÁ Z, KUNEŠ J: Plasma triglycerides and red cell ion transport alterations in genetically hypertensive rats. *Hypertension* **30**: 636-640, 1997.

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