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...
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).

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

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Pump (mmol/l erythrocytes/h)</th>
<th>Cotransport (mmol/l erythrocytes/h)</th>
<th>Leak (mmol/l erythrocytes/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=5)</td>
<td>8.515±0.367</td>
<td>2.493±0.358</td>
<td>9.480±0.488</td>
</tr>
<tr>
<td>Hypoxia (n=5)</td>
<td>7.920±0.359</td>
<td>2.424±0.254</td>
<td>9.047±0.640</td>
</tr>
</tbody>
</table>

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<thead>
<tr>
<th>Group</th>
<th>Pump (mmol/l erythrocytes/h)</th>
<th>Cotransport (mmol/l erythrocytes/h)</th>
<th>Leak (mmol/l erythrocytes/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=5)</td>
<td>10.669±0.640</td>
<td>0.707±0.052</td>
<td>0.815±0.057</td>
</tr>
<tr>
<td>Hypoxia (n=5)</td>
<td>9.992±0.677</td>
<td>0.456±0.078</td>
<td>0.823±0.061</td>
</tr>
</tbody>
</table>

* 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)

<table>
<thead>
<tr>
<th>Group</th>
<th>Pump (mmol/l erythrocytes/h)</th>
<th>Cotransport (mmol/l erythrocytes/h)</th>
<th>Leak (mmol/l erythrocytes/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=6)</td>
<td>6.191±0.357</td>
<td>1.825±0.365</td>
<td>4.596±0.339</td>
</tr>
<tr>
<td>Hypoxia (n=7)</td>
<td>5.570±0.249</td>
<td>1.857±0.308</td>
<td>4.589±0.150</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Pump (mmol/l erythrocytes/h)</th>
<th>Cotransport (mmol/l erythrocytes/h)</th>
<th>Leak (mmol/l erythrocytes/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=6)</td>
<td>5.455±0.244</td>
<td>1.561±0.171</td>
<td>0.487±0.012</td>
</tr>
<tr>
<td>Hypoxia (n=7)</td>
<td>5.028±0.288</td>
<td>1.598±0.221</td>
<td>0.442±0.018</td>
</tr>
</tbody>
</table>

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)
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.

Acknowledgements
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References


Reprint requests
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