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EFFECTS OF NITROGLYCERIN ON ENERGY METABOLISM OF RAT RETICULOCYTES

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Nitric oxide (NO) in many cells inactivates aconitase and mitochondrial respiratory chain, and influenced glyceraldehyde 3-phosphate dehydrogenase activity. The aim of this study was to evaluate role of nitroglycerin (NTG), a widely used NO donor, on energy metabolism of rat reticulocytes. Rat reticulocyte rich red blood cell suspensions containing 70—100% of reticulocytes, were aerobically incubated without (control) or in the presence of different concentrations of (a) NTG (0.1, 0.25, 0.5, 1.0, 1.5 mmol/l), (b) 8-Br-cGMP (0.1, 0.5, 1.0 mmol/l) and (c) NaNO₂ and NaNO₃ (1 mmol/l). NTG in dose- and time-dependent manner decreased total (p<0.05; EC₅₀ = 0.78 ± 0.05 mmol/l) and coupled (p<0.05; EC₅₀ = 0.50 ± 0.04 mmol/l) oxygen consumption (p<0.05; EC₅₀ = 0.36 ± 0.01 mmol/l). They were accompanied by stimulation of glycolysis, as measured by increased glucose consumption and lactate accumulation (p<0.01; EC₅₀ = 0.53 and 0.53 mmol/l, respectively). Levels of all glycolytic intermediates in the presence of NTG indicate stimulation of HK-PFK, GA3PDH and PK activity. NTG significantly decreased ATP level, which accompanied by increased ADP and AMP levels. However, level of total adenine nucleotides (TAN) was significantly lower, which was consequence of increased catabolism of adenine nucleotides (increased hypoxanthine level; p<0.05). Stimulation of glycolysis accompanied with inhibition of the Oxp, activation of HK-PFK, decrease of ATP and simultaneous rise of ADP and AMP levels, all together represent an example of Pasteur effect occurring in NTG-treated reticulocytes. In rat reticulocytes under steady state conditions 93% of overall energy was produced by Oxp, but only 7% by glycolysis. Due to decrease of coupled oxygen consumption in the presence of NTG, ATP production via Oxp was significantly diminished. Simultaneous increase of glycolytic ATP production is not enough to provide constant either ATP production or concentration. Calculated mean ATP-turnover time was prolonged even for 45% in the presence of 1.5 mmol/l NTG. Metabolic effects of NTG were not mimic by exogenous 8-Br-cGMP, NaNO₂ or NaNO₃, which indicate that NTG induced a) inhibition of coupled respiration and b) stimulation of glycolysis in rat reticulocytes are mediated by NO as an effector molecule.

Key words: rat reticulocytes; energy metabolism, oxidative phosphorylation, glycolysis, nitric oxide, nitroglycerin
INTRODUCTION

More recently, organic nitrates experienced a remarkable revival with the finding that they have to be regarded as prodrugs acting via the release of nitric oxide (NO), a discovery which coincided with the demonstration that endothelium-derived relaxing factor (EDRF) is chemically identical with NO (1). NO is a small hydrophobic molecule with chemical properties that make it uniquely suitable as both an intra- and intercellular messenger. The primary reactions of NO are almost exclusively limited to other species possessing unpaired electrons, such as the iron in haeme proteins, as well as nonhaeme iron (proteins with iron-sulfur centers), thiols, molecular oxygen and superoxide (2, 3).

Glycolysis is the only energy producing process in mammalian erythrocytes, while in reticulocytes energy is provided by glycolysis (10%), as well as, by oxidative phosphorylation (90%) (4). Nitric oxide in many cells inactivates aconitase and mitochondrial respiratory chain at the complex I and II (proteins with iron-sulfur centers) (5—7) and cytochrome oxidase (haeme protein) (8—12). NO at a low concentrations can potently deenergize isolated liver, brain (13) and myocardial mitochondria (14) at oxygen concentrations that prevail in cells and tissues. In addition, NO causes ADP-ribosylation and inhibition of glyceraldehyde 3-phosphate dehydrogenase — GA3PDH, a glycolytic enzyme (15—17). Contrary of these results Mallozzi and coworkers (18, 19) showed NO-induced phosphorylation of Band 3 and consequently activation of GA3PDH in erythrocytes.

However, there are no data concerning the effects of NO on energy metabolism of red blood cells, especially regarding their high content of haemoglobin — an effective scavenger of NO (20, 21). Therefore the aim of this study was to investigate the role of nitroglycerin (NTG), a widely used donor of NO (22, 23), on rat reticulocytes energy metabolism.

MATERIALS AND METHODS

In this study reticulocyte-rich red blood cell suspensions of rats (Wistar albino rats of 250—350 g body mass) were used. Reticulocytosis was induced by phenylhydrazine hydrochloride treatment (35 mg/kg body weight during three days) (24). After 7—8 days, when blood was taken by exsanguination, reticulocytes amounted to 70—100%. Three times washed red blood cells were resuspend in incubation buffer containing: 50 mmol/l Hapes, 100 mmol/l NaCl, 1 mmol/l MgCl2, 1 mmol/l Na2PO4, 5 mmol/l glucose and 2 mmol/l CaCl2, pH 7.4 at 37°C (24). Cell suspensions (final haematocrit value about 0.20) were incubated 2 hours, aerobically without (control) or in the presence of different concentrations of (a) NTG (Zorka, Šabac, Yugoslavia): 0.1, 0.25, 0.5, 1.0 and 1.5 mmol/l; (b) 8-Br-cGMP (Boehringer, Mannheim, Germany): 0.1, 0.5 and 1.0 mmol/l; (c) NaNO2 and NaNO3 (Alkaloid, Skopje, Macedonia): 1 mmol/l. Chemicals for solutions were obtained from Sigma (St. Louis, MO, USA) and Merck (Darmstadt, Germany) while all enzymes were Boehringer (Mannheim, Germany) products.
Oxygen consumption was measured by Warburg technique (25). Coupled oxygen consumption (the part of total oxygen consumption used for ATP production in oxidative phosphorylation), was calculated as the difference between total and oligomycin (5 μmol/l) resistant oxygen consumption (26). On the basis of coupled oxygen consumption and P/O ratio 2.5 amount of ATP produced by OxP was calculated (27). Glycolytic energy production was calculated on the basis of lactate/ATP ratio of 1 (27). Total energy production in reticulocytes was calculated by addition of glycolytic to mitochondrial ATP production. ATP-turnover calculated on the basis of total ATP production and ATP concentration (27).

The aliquots of red blood cell suspensions for extraction of glucose, lactate, glycolytic intermediates, adenine nucleotides and hypoxanthine taken at the start and after two hours of aerobic incubation. Extraction was done with 1 vol of ice-cold 0.6 mol/l perchloric acid and extracts were neutralized with 0.25 vol of 1 mol/l TRA — 2.3 mol/l K₂CO₃. In neutralized perchloric acid extracts glucose, lactate, glycolytic intermediates (glycerate 2,3-diphosphate = 2,3-DPG, glyceraldehyde 3-phosphate = 3PG, glyceraldehyde 2-phosphate = 2PG, phosphoenolpyruvate = PEP, pyruvate), adenine nucleotides (ATP, ADP, AMP) and hypoxanthine were determined enzymatically by means of spectrophotometric technique (28–34). The rest of glycolytic intermediates (glucose 6-phosphate = G6P, fructose 6-phosphate = F6P, fructose 1,6-diphosphate = FDP, dihydroxyacetone phosphate and glyceraldehyde 3-phosphate as triosephosphates = TP) were determined enzymatically by means of spectrofluorometric technique (35).

All values are expressed as mean±SEM. Statistical evaluation was performed by Student’s t-test for paired observations. For all comparisons p<0.05 was regarded as significant. The concentration of NTG that produces a response in 50% of the maximal effect (EC50) was evaluated by linear regression in dose-response manner by the method of Bowman and Rand (36).

RESULTS

Results of our investigations show that total, coupled and uncoupled oxygen consumption in reticulocyte-rich red blood cell suspensions amount to 31.26±2.33, 21.97±1.53 and 9.33±0.89 μmol/ml reticulocytes/2 h, respectively (Fig. 1), which is in accordance with our previous results (24). Effects of NTG appear to be dependent on the time of incubation and dose of NTG: significant reduction of coupled (p<0.05) and elevation of uncoupled oxygen consumption (p<0.05) occurs after 30 min of incubation in the presence of 1.0, 1.5 and 0.5, 1.0, 1.5 mmol/l NTG, respectively (Fig. 1). This is results as slight (no significant), dose-dependent reduction of total oxygen consumption (p>0.05; Fig. 1). The concentration of NTG that produces a response in 50% of the maximal effect (EC50) on reticulocyte respiration (values showed in Fig. 1) indicate that uncoupled and coupled oxygen consumption were more sensitive to NTG influence than total oxygen consumption.

Reduction of coupled oxygen consumption indicates reduction of energy production (ATP) in the oxidative phosphorylation (OxP). It is accompanied by stimulation of glycolysis, which represents an example of Pasteur effect (4). Namely, glucose consumption and lactate accumulation during 2 hours of aerobic incubation (5.04±1.00 and 7.94±1.34 μmol/ml cells/2 h, respectively;
Fig. 1. Effects of NTG on reticulocyte respiration. EC50 values in 30, 60, 90 and 120 min are for total: 0.97, 0.77, 0.73, 0.67; coupled: 0.64, 0.47, 0.47, 0.44 and uncoupled oxygen consumption: 0.37, 0.32, 0.37, 0.39 mmol/l NTG, respectively. Each point with a bar represents the mean ± SEM for 4–6 paired experiments. Values in μmol/ml rtics. *p < 0.05, **p < 0.02, ***p < 0.01, ****p < 0.001, control (0 mmol/l NTG) versus NTG (the other concentrations).
Table 1. Effects of NTG on glycolytic intermediate levels in rat reticulocytes.

<table>
<thead>
<tr>
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<th>0</th>
<th>0.1</th>
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<tbody>
<tr>
<td>G6P</td>
<td>278.6 ± 32.2</td>
<td>284.1 ± 58.1</td>
<td>275.8 ± 33.2</td>
<td>208.9 ± 28.6</td>
<td>189.4 ± 23.7*</td>
<td>130.9 ± 13.1*</td>
</tr>
<tr>
<td>F6P</td>
<td>60.1 ± 8.8</td>
<td>59.5 ± 19.5</td>
<td>80.6 ± 7.7</td>
<td>43.3 ± 5.7</td>
<td>40.3 ± 8.9</td>
<td>30.1 ± 3.0*</td>
</tr>
<tr>
<td>FDP</td>
<td>28.7 ± 5.8</td>
<td>16.9 ± 3.6</td>
<td>13.8 ± 4.0</td>
<td>20.3 ± 5.4</td>
<td>18.6 ± 6.4</td>
<td>8.1 ± 0.8*</td>
</tr>
<tr>
<td>TP</td>
<td>66.4 ± 13.5</td>
<td>59.8 ± 19.4</td>
<td>35.2 ± 13.7</td>
<td>23.3 ± 7.3</td>
<td>50.5 ± 9.0</td>
<td>46.5 ± 9.4</td>
</tr>
<tr>
<td>2,3-DPG</td>
<td>11.1 ± 0.4</td>
<td>13.2 ± 1.6</td>
<td>10.8 ± 2.0</td>
<td>9.8 ± 1.7</td>
<td>9.9 ± 0.6</td>
<td>10.0 ± 0.3</td>
</tr>
<tr>
<td>3PG</td>
<td>63.6 ± 13.4</td>
<td>66.8 ± 10.0</td>
<td>78.9 ± 12.8</td>
<td>50.2 ± 17.7</td>
<td>82.0 ± 25.8</td>
<td>50.9 ± 5.1</td>
</tr>
<tr>
<td>2PG</td>
<td>16.2 ± 2.7</td>
<td>21.2 ± 7.2</td>
<td>25.9 ± 5.7</td>
<td>20.2 ± 3.3</td>
<td>20.4 ± 4.6</td>
<td>22.7 ± 4.2</td>
</tr>
<tr>
<td>PEP</td>
<td>83.9 ± 24.8</td>
<td>99.0 ± 41.0</td>
<td>59.6 ± 8.0</td>
<td>64.6 ± 12.7</td>
<td>71.3 ± 18.9</td>
<td>67.1 ± 10.1</td>
</tr>
<tr>
<td>PYR</td>
<td>248 ± 8</td>
<td>243 ± 40</td>
<td>496 ± 50*</td>
<td>1228 ± 20***</td>
<td>1781 ± 67****</td>
<td>4680 ± 237****</td>
</tr>
<tr>
<td>LAC</td>
<td>8.08 ± 0.42</td>
<td>9.29 ± 1.60</td>
<td>9.45 ± 0.77</td>
<td>10.34 ± 1.51</td>
<td>12.12 ± 0.88***</td>
<td>16.56 ± 1.16****</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM for 4–6 paired experiments. Values for all glycolytic intermediates are in mmol/ml cells, except for 2,3-DPG and LAC in µmol/ml cells.

*p < 0.05, ***p < 0.01, ****p < 0.001, control (0 mmol/l NTG) versus NTG (the other concentrations).

Fig. 2) were significantly increased in the presence of 0.5, 1.0 and 1.5 mmol/l NTG (p < 0.01). In addition, EC50 of NTG for coupled oxygen consumption (value represent mean of EC50 ± SEM for all time) and glycolysis (0.50 ± 0.04 and 0.53 mmol/l, respectively) indicate that stimulation of glycolysis was consequence of OxP inhibition by NTG. Levels of all glycolytic intermediates were showed in Table 1.

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**Fig. 2.** Effects of NTG on glucose consumption and lactate accumulation in rat reticulocytes after 2 hours of aerobic incubation. EC50 values for glucose consumption and lactate accumulation are: 0.53 and 0.53 mmol/l NTG, respectively. Each point with a bar represents the mean ± SEM for 4–6 paired experiments. Values in µmol/ml cells. *p < 0.05, ***p < 0.01, control (0 mmol/l NTG) versus NTG (the other concentrations).
Decreased levels of G6P, F6P, FDP (p < 0.05) and triosephosphates (TP; p > 0.05), as well as, increased levels of pyruvate in the presence of NTG indicate stimulation hexokinase — phosphofructokinase (HK–PFK), glyceraldehyde 3-phosphate dehydrogenase (GA3PDH) and pyruvate kinase (PK) activity.

Besides reduction of cell respiration and stimulation of glycolysis, NTG induces marked alterations of energy status of reticulocytes. Dose-dependent decrease of ATP level (p < 0.05) was accompanied by elevation of ADP and AMP levels (p < 0.05; Tab. 2). However, level of total adenine nucleotides (TAN) was significantly lower in the presence of NTG (p < 0.02; Tab. 2). Because NTG-induced decrease of ATP and TAN levels can be the consequence of increased catabolism of adenine nucleotides, we determined concentration of hypoxanthine, a final product of adenine nucleotide catabolism in red blood cells (37). Hypoxanthine level increases in dose-dependent manner in the presence of NTG. This elevation is sufficient to compensate decrease of TAN level (Tab. 2).

Table 2. Effects of NTG on adenine nucleotides (ATP, ADP, AMP) and hypoxanthine levels in rat reticulocytes.

<table>
<thead>
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<th>0</th>
<th>0.1</th>
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<th>0.5</th>
<th>1.0</th>
<th>1.5</th>
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</thead>
<tbody>
<tr>
<td>ATP</td>
<td>1.51 ± 0.12</td>
<td>1.37 ± 0.12</td>
<td>1.48 ± 0.13</td>
<td>1.36 ± 0.15</td>
<td>1.14 ± 0.09*</td>
<td>0.83 ± 0.06***</td>
</tr>
<tr>
<td>ADP</td>
<td>128 ± 15</td>
<td>127 ± 8</td>
<td>166 ± 19</td>
<td>160 ± 19</td>
<td>153 ± 10</td>
<td>281 ± 31*</td>
</tr>
<tr>
<td>AMP</td>
<td>43 ± 8</td>
<td>74 ± 6</td>
<td>63 ± 2</td>
<td>61 ± 14</td>
<td>73 ± 15</td>
<td>185 ± 28*</td>
</tr>
<tr>
<td>TAN</td>
<td>1.68 ± 0.13</td>
<td>1.51 ± 0.14</td>
<td>1.71 ± 0.13</td>
<td>1.51 ± 0.16</td>
<td>1.35 ± 0.10</td>
<td>1.24 ± 0.08**</td>
</tr>
<tr>
<td>HX</td>
<td>0.33 ± 0.02</td>
<td>0.38 ± 0.03</td>
<td>0.40 ± 0.04</td>
<td>0.43 ± 0.01</td>
<td>0.50 ± 0.07</td>
<td>0.62 ± 0.09*</td>
</tr>
<tr>
<td>TAN+HX</td>
<td>1.94 ± 0.13</td>
<td>1.80 ± 0.27</td>
<td>2.01 ± 0.20</td>
<td>1.65 ± 0.24</td>
<td>1.89 ± 0.08</td>
<td>1.78 ± 0.12</td>
</tr>
</tbody>
</table>

TAN — total adenine nucleotides; HX — hypoxanthine; Values represent mean ± SEM for 4—6 paired experiments. Values for ATP, TAN, HX and TAN+HX are in μmol/ml cells, while for ADP and AMP in mmol/ml cells.

*p < 0.05. **p < 0.02. ***p < 0.01, control (0 mmol/l NTG) versus NTG (the other concentrations).

In rat reticulocytes under steady state conditions, according to the results of this work, 93% of overall energy was produced by OxP (54.45 ± 5.3 μmol ATP/ml retcs/h), but only 7% by glycolysis (3.97 ± 0.67 μmol ATP/ml retcs/h) (Tab. 3). The ATP level was estimated to be 1.51 ± 0.12 μmol/ml reticulocytes and a mean turnover time of 1.54 was calculated (Tab. 3). Due to decrease of coupled oxygen consumption in the presence of NTG, ATP production via OxP was significantly diminished (p < 0.01; EC50 = 0.47 mmol/l). Simultaneous increase of glycolytic ATP production (p < 0.01; EC50 = 0.53 mmol/l) is not enough to provide constant either ATP production or concentration. Calculated mean ATP-turnover time was prolonged even for 45% in the presence of 1.5 mmol/l NTG (Tab. 3), which indicates an inhibition of ATP-consuming processes in NTG-treated reticulocytes.
Table 3. Effects of NTG on energy production, ATP concentration and ATP-turnover in rat reticulocytes.

<table>
<thead>
<tr>
<th>NTG (mmol/l)</th>
<th>OxP ATP prod µmol/ml cells/h</th>
<th>Glyc ATP prod µmol/ml cells/h</th>
<th>Total ATP prod µmol/ml cells/h</th>
<th>ATP µmol/ml cells</th>
<th>ATP turnover times/h</th>
<th>ATP turnover min</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>54.45±5.30</td>
<td>3.97±0.67</td>
<td>58.42±2.98</td>
<td>1.51±0.12</td>
<td>38.69</td>
<td>1.55</td>
</tr>
<tr>
<td>0.10</td>
<td>49.55±10.70</td>
<td>4.72±0.68</td>
<td>54.27±5.69</td>
<td>1.33±0.12</td>
<td>40.80</td>
<td>1.47</td>
</tr>
<tr>
<td>0.25</td>
<td>47.90±3.90</td>
<td>4.96±0.19</td>
<td>52.86±2.04</td>
<td>1.48±0.13</td>
<td>35.72</td>
<td>1.68</td>
</tr>
<tr>
<td>0.50</td>
<td>35.95±6.45</td>
<td>5.87±0.19***</td>
<td>41.82±3.32***</td>
<td>1.36±0.15</td>
<td>30.75</td>
<td>1.95</td>
</tr>
<tr>
<td>1.00</td>
<td>25.60±5.70***</td>
<td>7.46±0.46*</td>
<td>33.06±3.08****</td>
<td>1.14±0.09*</td>
<td>29.00</td>
<td>2.07</td>
</tr>
<tr>
<td>1.50</td>
<td>13.60±5.25***</td>
<td>9.60±0.59***</td>
<td>23.20±2.92****</td>
<td>0.83±0.06***</td>
<td>27.95</td>
<td>2.15</td>
</tr>
<tr>
<td>EC50</td>
<td>0.47</td>
<td>0.53</td>
<td>0.46</td>
<td>0.63</td>
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</tr>
</tbody>
</table>

ATP production in oxidative phosphorylation (OxP ATP prod) was calculated on the basis of P/O = 2.5, and in glycolysis (Glyc ATP prod) on the basis of lactate/ATP = 1 (27). Values for EC50 are in mmol/l NTG.

Values represent mean ± SEM for 4–6 paired experiments.

*p<0.05, ***p<0.01, ****p<0.001, control (0 mmol/l NTG) versus NTG (the other concentrations).

8-Br-cGMP only in high concentration (1 mmol/l) significantly decreased total and coupled oxygen consumption (p<0.05; Fig. 3), while glycolysis unaltered under its influences (data not shown). NaNO₂ and NaNO₃ did not alter OxP and glycolysis in rat reticulocytes (data not shown).

![oxygen consumption graph](image)

*Fig. 3. Effects of 8-Br-cGMP on reticulocyte respiration after 2 hours of aerobic incubation. Each point with a bar represents the mean ± SEM for 3 paired experiments. Values in µmol/ml rcts.

*p<0.05, control (0 mmol/l 8-Br-cGMP) versus 8-Br-cGMP (the other concentrations).
DISCUSSION

In this work we analyzed effects of nitroglycerin on energy metabolism of rat reticulocytes. Our interest for oxidative phosphorylation (OxP) and glycolysis, a processes including in ATP production of reticulocytes, is based on the results of the other authors, which showed NO-induced alteration of OxP and glycolysis. Namely, nitric oxide in many cells inactivates aconitase and mitochondrial respiratory chain (at the complex I, II and cytochrome oxidase) (5—12) and induced alteration in glyceraldehyde 3-phosphate dehydrogenase (GA3PDH) activity (15—19). Results of our investigations showed that reticulocyte respiration was significantly altered by NTG in dose- and time-dependent manner: decrease of coupled oxygen consumption (EC50 = 0.50 ± 0.04 mmol/l) and increase of uncoupled oxygen consumption (EC50 = 0.36 ± 0.01 mmol/l). Inhibition of coupled oxygen consumption was probably consequence of NO-induced inhibition of cytochrome oxidase (8—10). However, free oxygen radicals are generated due to mitochondrial activity (38—40). In addition, NO induced superoxide anion radical (O_2^-) generation in mitochondria (41, 42). Hence, NO liberated from NTG react with O_2^- to produce peroxynitrite (ONOO^-) (3), which influenced ATP-synthase (43) and probably contributed to inhibition of ATP production in OxP. Our results were in accordance with results presented by Richter and coworkers (13, 44), which indicate that NO at a low concentrations can potently deenergize isolated liver and brain mitochondria at oxygen concentrations that prevail in cells and tissues.

Elevation of uncoupled oxygen consumption was probably consequence of increased processes of oxidation in reticulocytes (4) under influence of NO as free radical compound.

Reduction of coupled oxygen production was accompanied by stimulation of glycolysis, as measured by lactate accumulation and glucose consumption. However, even 2.4-fold stimulation of reticulocyte glycolysis (in the presence of 1.5 mmol/l NTG), providing 43% of whole energy production, was not sufficient to compensate decreased energy production due to inhibition of OxP. What is the reason for the stimulation of glycolysis in NTG-treated reticulocytes? Levels of all glycolytic intermediates and application of "cross-over" theorem (4) indicate stimulation HK–PFK, GA3PDH and PK activity. Stimulation of glycolysis accompanied with inhibition of the OxP, activation of HK–PFK, decrease of ATP and simultaneous rise of ADP and AMP levels, all together represent an example of Pasteur effect (4) occurring in NTG-treated reticulocytes. However, in mature erythrocytes NTG dose-dependently increased glycolytic rate and decreased ATP level (Maeltić and Kostić, unpublished data), which was in accordance with results showed by Mallozzi and coworkers (18, 19) that indicate NO-induced phosphorylation
of Band 3 and consequently stimulation of GA3PDH activity. These data indicate that NTG itself stimulates glycolysis, but not only through the Pasteur effect which could not appear in mature erythrocytes. Therefore our data have showed that NO released by NTG stimulated glycolytic sequence, while Dimmeler, Brüne and coworkers (15—17) showed NO-induced ADP-ribosylation and inhibition of GA3PDH.

Besides lower ATP production, a dose-dependent decrease ATP level in the presence of NTG was also found. However, increase of ADP and particularly AMP content did not sufficient to prevent the loss of ATP, which occurred when ATP production by OxP inhibited by NTG. Our results showed that decreased levels of adenine nucleotides were consequence of increased catabolism of adenine nucleotides.

In rat reticulocytes under steady state conditions, according to the results of this work, 93% of overall energy was produced by OxP, but only 7% by glycolysis. Due to decrease of coupled oxygen consumption in the presence of NTG, ATP production via OxP was significantly diminished. Simultaneous increase of glycolytic ATP production is not enough to provide constant either ATP production or concentration. Calculated mean ATP-turnover time was prolonged even for 45% in the presence of 1.5 mmol/l NTG, which indicates an inhibition of ATP-consuming processes (the globin synthesis, Na,K-ATPase activity, proteolysis (4, 45, 46)) in NTG-treated reticulocytes.

Results presented in this study were exactly showed NTG (NO)-induced alterations in energy metabolism of rat reticulocytes. However, reticulocytes have high content of haemoglobin, an effective scavenger of NO (20, 21). Wennmalm and coworkers (21) showed that plasma or whole venous or arterialized blood from healthy human donors incubated with NO resulting in formation of methaemoglobin, nitrosyl haemoglobin and nitrite and nitrate, an inactivated forms of NO. However, under physiological conditions NO react with SH groups of haemoglobin to form S-nitrosohaemoglobin (3, 47—49). There was possibility that NO liberated from NTG react with haemoglobin and produced S-nitrosohaemoglobin, which liberated NO and preserved NO-induced effects on energy metabolism of rat reticulocytes.

On the other hand, metabolic effects of NTG were not mimic by 8-Br-cGMP (exogenous analogue of cGMP, a second messenger of NO (50—52)), except the slight, dose independent inhibition of OxP accompanied by no changes of glycolytic rate. However, Xie and coworkers (53) showed that NO and 8-Br-cGMP influenced OxP by two different mechanisms. Exogenous NaN02 and NaN03 (nitrates and nitrates were secondary products of NO in aerobic aqueous solutions (54—56)), did not alter OxP and glycolysis in rat reticulocytes. In addition, reticulocytes synthesized cytochrome P450 (57) and glutathione-S-transpherase (58—60), enzymes that were included in biotransformation of NTG to active compound of nitric oxide (61, 62).
On the basis of the implications presented in this study we concluded that NTG-induced a) inhibition of coupled and stimulation of uncoupled respiration, b) stimulation of glycolysis, c) increased catabolism of adenine nucleotides, d) decreased ATP production and concentration and e) prolonged ATP-turnover in rat reticulocytes were mediated by NO as an effector molecule.

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