FEBS Letters 582 (2008) 1809–1815

Maintenance of luminal NADPH in the endoplasmic reticulum promotes the survival of human neutrophil granulocytes

Tamás Kardon[a,1], Silvia Senesi[b,1], Paola Marcolongo[b], Balázs Legeza[a], Gábor Bánhegyi[a,b,*], József Mandl[a], Rosella Fulceri[b], Angelo Benedetti[b]

[a] *Semmelweis Egyetem Orvosi Vegytani, Molekuláris Biológiai és Patobiokémiai Intézete & MTA-SE Patobiokémiai Kutatócsoport, Budapest, Hungary*
[b] *Dipartimento di Fisiopatologia, Medicina Sperimentale e Sanità Pubblica, Università di Siena, Viale Aldo Moro 53100, Siena, Italy*

Received 18 March 2008; revised 21 April 2008; accepted 27 April 2008

Available online 8 May 2008

Edited by Robert Barouki

Abstract The present study demonstrates the expression of hexose-6-phosphate dehydrogenase and 11β-hydroxysteroid dehydrogenase type 1 in human neutrophils, and the presence and activity of these enzymes in the microsomal fraction of the cells. Their concerted action together with the previously described glucose-6-phosphate transporter is responsible for cortisone–cortisol interconversion detected in human neutrophils. Furthermore, the results suggest that luminal NADPH generation by the cortisol dehydrogenase activity of 11β-hydroxysteroid dehydrogenase type 1 prevents neutrophil apoptosis provoked by the inhibition of the glucose-6-phosphate transporter. In conclusion, the maintenance of the luminal NADPH pool is an important antiapoptotic factor in neutrophil granulocytes.

Keywords: Apoptosis; 11β-Hydroxysteroid dehydrogenase type 1; Hexose-6-phosphate dehydrogenase; Glucose-6-phosphate transporter; Glucocorticoid

1. Introduction

The glucose 6-phosphate transporter (G6PT) of the endoplasmic reticulum (ER) allows the entry of the cytosolic metabolite glucose-6-phosphate (G6P) into the luminal space. G6P is the substrate for at least two luminal enzymes: glucose-6-phosphatase (G6Pase) and hexose-6-phosphate dehydrogenase (H6PDH). The activity of the former enzyme is crucial for the maintenance of blood glucose level [1,2]. Recent evidence show that H6PDH has a role in regulating the redox state of an ER pool of pyridine nucleotides, as well as in supplying the cofactor NADPH to 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) [3–7]. This enzyme, highly represented in liver and adipose tissue, is located within the ER lumen and plays a key role in the prereceptorial activation of glucocorticoids [8–10].

The genetic deficiency of G6PT, named glycogen storage disease type 1b (GSD 1b), causes – in addition to an inventory of symptoms ultimately due to the lack of G6Pase activity – a severe pathology of neutrophils/monocytes [11–14]. A recent report shows that G6PT knock-out mice also present with defects of neutrophils/monocytes largely mimicking those of GSD 1b patients, such as alterations of cell differentiation and growth, neutropenia, impairment of superoxide anion generation and chemotaxis [12]. We have previously observed that the chemical ablation of G6PT activity (with the selective inhibitor S3483) [15,16] results in an impaired differentiation of the promyelocytic HL-60 cell line, as well as in apoptosis of differentiated HL-60 cells and human neutrophils (PMNs) [17]. Consistently, other authors observed that apoptotic PMNs are present in the peripheral blood of GSD 1b patients [18].

In our aforementioned study [17] it was also observed that the PMN apoptosis induced by S3483 is fully prevented by the antioxidant vitamin E analogue, trolox C, as well as by the inhibitor of NADPH oxidase, diphenylene iodonium. On this ground, we hypothesized that G6P entry into the ER compartment mediated by G6PT might be required for the sufficient antioxidant protection of PMNs. The G6PT-dependent H6PDH activity in other tissues (i) keeps luminal pyridine nucleotides in reduced state and (ii) allows the reductase activity of 11β-HSD1. However, neither H6PDH nor 11β-HSD1 has been reported to be present and functional in the neutrophil ER. Therefore, experiments were undertaken to investigate the expression and activity of these enzymes in the ER of human PMNs, with special emphasis on the possibility of modulating neutrophil apoptosis through their activity.

2. Materials and methods

2.1. Preparation of human PMNs

Human PMN cells of healthy volunteers were isolated from peripheral venous blood collected in tubes containing 16 IU heparin/ml blood. The neutrophils were separated by dextran sedimentation method according to Hjorth et al. [19] with small modifications as described in details earlier [17]. Preparations contained more than 95% PMNs and cell viability (evaluated by trypan blue exclusion method) exceeded 97%.

2.2. Preparation of microsomal fractions

Microsomes from PMNs and rat liver were prepared as previously reported [17]. Human liver microsomes were prepared as detailed earlier [20]. The Ethical Committee of the Semmelweis University

*Corresponding author. Address: Dipartimento di Fisiopatologia e, Medicina Sperimentale e Sanità Pubblica, Università di Siena, Viale Aldo Moro 53100, Siena, Italy. Fax: +39 (0)577 234009.
E-mail address: banhegyi@unisi.it (G. Bánhegyi).

[1]These authors contributed equally to the work.

0014-5793/$34.00
doi:10.1016/j.febslet.2008.04.045

approved the study on the H6PDH/11βHSD1 in human liver specimens. Microsomes were resuspended in MOPS-KCl buffer (100 mM KCl, 20 mM NaCl, 1 mM $MgCl_2$ and 20 mM MOPS) pH 7.2 including a cocktail of protease inhibitors, and maintained under liquid N_2 until used. Immediately before Western blot analysis or enzyme assay, the possible cytosolic or other contaminants loosely associated with the vesicles were removed by a rapid washing as follows: polyethylene glycol (at 7% w/v final concentration) was added to the microsomes and the suspension was centrifuged at $4000 \times g$ for 15 s. After discarding the supernatant, the microsomal pellet was resuspended in polyethylene-glycol-free MOPS-KCl buffer [21]. The microsomal fraction was enriched in ER-specific proteins (calnexin, IP_3R3, SERCA2b) as revealed by Western blotting (data not shown).

2.3. Western blot

Microsomal proteins were loaded on polyacrylamide gels and blotted on nitrocellulose. Immunoblots were probed with a rabbit polyclonal antiserum against the lactonase domain (residues 539-791) of human H6PDH kindly provided by Dr. E. van Schaftingen (ICP, Bruxelles, Belgium), or with rabbit polyclonal antibodies to mouse 11βHSD1 (Alpha Diagnostic International). After reacting with the secondary antibodies, blots were analyzed either by enhanced chemiluminescence or using fluorescent secondary antibodies (Amersham Biosciences). The blots were visualized by Typhoon 8600 multiimager (Amersham Biosciences).

2.4. RT-PCR

PMN RNA was isolated using the RNeasy Mini Kit (Qiagen) and human liver total RNA was from Biocat GmbH. 500 ng of RNA was reverse transcribed by using the SuperScript® II First-Strand Synthesis System (Invitrogen). For H6PDH, the oligonucleotide primers were: sense, 5′-CAACTGGGGACCTGGCTAAGAAGT-3′; antisense, 5′-GTTGATGAGAGGCAGGCTAAGGCT 3′. For 11βHSD1 the oligonucleotide primers were: sense, 5′-GAACATCAATAA AAAGAAGTCAGA-3′; antisense, 5′-CATTATTATTACATTTC-CATTTTG-3′. PCR products were separated on a 1% agarose gel. The identity of the RT-PCR products was confirmed by DNA sequencing.

2.5. Enzyme assays

H6PDH and isocitrate dehydrogenase activities were evaluated by measuring NADPH formation upon the addition of 1 mM $NADP^+$ and either 10 μM glucose-6-phosphate or 1 mM isocitrate to microsomes. Limited access of the cofactor compounds to the intraluminal enzymes proved the intactness of the microsomes. Microsomes have been subsequently permeabilized with Triton X-100 (1% final concentration) to allow the free access of the cofactor to the intraluminal enzyme. The product of lactonase activity, 6-phosphogluconic acid, was then measured enzymatically with 6-phosphogluconate dehydrogenase on the basis of NADPH formation. 11βHSD1 activity was measured in both directions, as cortisone reductase and cortisol dehydrogenase activity, upon the addition of 100 μM NADPH or $NADP^+$ and 20 μM cortisone or cortisol to microsomes. Microsomes have been subsequently permeabilized with Triton X-100 (1% final concentration) to allow the free access of the cofactors to the intraluminal enzyme. NADPH fluorescence was monitored at 350 nm excitation and 460 nm emission wavelengths by using a Cary Eclipse fluorescence spectrophotometer (Varian).

2.6. Evaluation of apoptosis

Apoptosis was evaluated by annexin–propidium iodide staining as described in details earlier [17].

3. Results

3.1. H6PDH in microsomes of human neutrophils

H6PDH is expressed in human PMNs both at mRNA and protein level. As shown in Fig. 1A, the analysis of RT-PCR products showed a band whose apparent size (2094 bp) is consistent with a mRNA coding for a protein of approximately 90 kDa, which is the M_r of the ER H6PDH protein; human liver RNA gave the same band [22]. Accordingly, in Western blot analysis of microsomal proteins from PMNs, a band at an apparent M_r of ≈90 kDa was immunorevealed by antibodies towards the lactonase domain of H6PDH; this band was also present in human (and rat) liver microsomes (Fig. 1B).

Fig. 1C shows that H6PDH activity was also present in PMN microsomes. In the presence of $NADP^+$, a marked G6P-dependent NADPH formation was evident upon permeabilization of the microsomal membrane (addition of Triton X-100, see arrow), whilst a little activity was only present before permeabilization. This was expected since $NADP^+$ cannot easily cross the ER membrane, and indicates the predicted luminal compartmentation of the enzyme [22,23]. The ER H6PDH is a dual enzyme possessing both G6P dehydrogenase and 6-phosphogluconolactonase activity [24]. Therefore, 6-phosphogluconolactone – derived from G6P oxidation – should be further metabolized by the same enzyme to 6-phosphogluconate. The latter metabolite was indeed formed, as revealed by the fact that the addition of 6-phosphogluconic acid dehydrogenase to microsomal incubates, in which G6P has been already oxidized to 6-phosphogluconolactone, resulted in a further increase in NADPH level (Fig. 1C). The amount of NADPH produced in the latter reaction (i.e., the dehydrogenation of 6-phosphogluconic acid) was roughly the same as NADPH amount derived from G6P oxidation (see Fig. 1C). This indicates that 6-phosphogluconate accumulates in the lumen, possibly because the downstream enzymes of the pentose pathway are not or poorly represented in the microsomes.

Since the presence of isocitrate dehydrogenase, another NADPH generating enzyme was reported in liver and adipose tissue microsomes [21], an attempt was made to demonstrate this activity in PMN microsomes. Very low $NADP^+$-dependent latent isocitrate dehydrogenase activity was detected (less than 5% of the observed H6PDH activity).

3.2. 11βHSD1 in microsomes of human neutrophils

Not only H6PDH, but also 11βHSD1 is represented in the ER of PMNs. The enzyme was expressed both at the mRNA and the protein level, as shown in Fig. 2A and B, respectively. Accordingly, 11βHSD1 activity could be also revealed in PMN microsomes. Upon the addition of cortisone or cortisol to permeabilized microsomes, NADPH was consumed or $NADP^+$ was reduced to NADPH, respectively (Fig. 2C). The activities appeared upon permeabilization of the microsomal membrane only. This is consistent with the luminal compartmentation of 11βHSD1 activity, as in the case of H6PDH [25,26].

3.3. Cortisol prevents the proapoptotic effect of the inhibition of microsomal G6P transport in human PMNs

As we have already shown, the addition of the G6PT inhibitor S3483 induced apoptosis in human granulocytes (Fig. 3). Addition of cortisol at high (20 μM) concentration did not alter the rate of apoptosis. Under these circumstances 11βHSD1 presumably acts as a dehydrogenase and generates NADPH in the ER lumen. When S3483 and cortisol were added together, cortisol significantly reduced the proapoptotic effect of the G6PT inhibitor. Inhibition of 11βHSD1 activity with carbenoxolone completely abolished the preventive effect of cortisol (data not shown). Physiological cortisol concentrations (20–100 nM) were ineffective. Cortisone addition did not increase

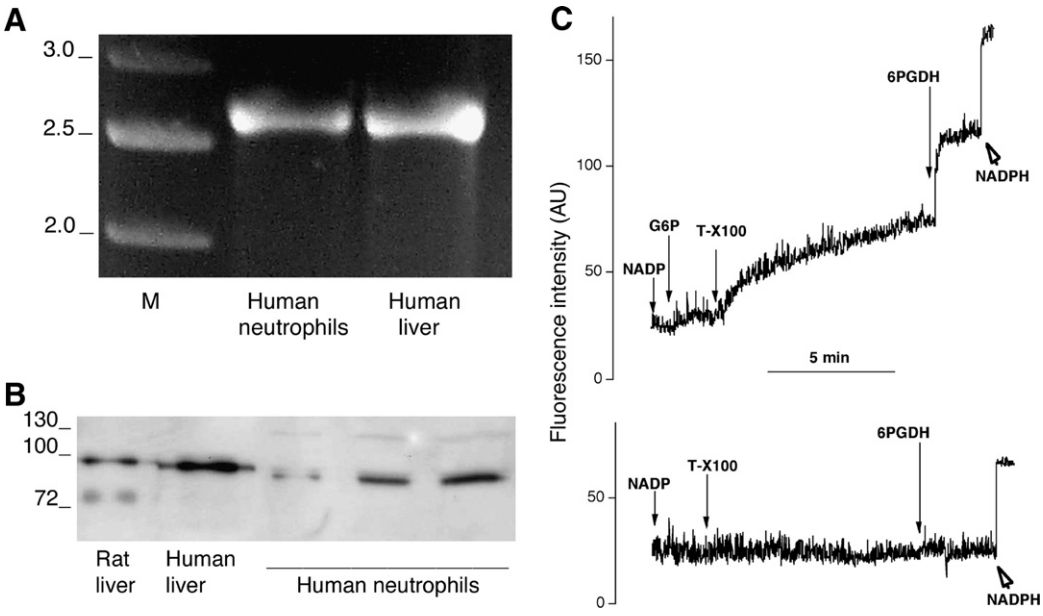

Fig. 1. Expression of H6PDH at mRNA (A) and protein (B) level and H6PDH activity (C) in human PMNs. (A) Total RNA isolated from human neutrophils and human hepatic tissue (as a control) was reverse transcribed and subjected to PCR. A representative electrophoresis of the PCR products (revealed with ethidium bromide) of three is shown. The first lane (M) contains a mixture of DNA fragments of different size (in kilobase pairs). (B) Microsomes were prepared from human granulocytes and human or rat hepatic tissue (as controls), and microsomal proteins were separated by 8% SDS–PAGE. Gels were blotted on a nitrocellulose membrane and the H6PDH protein was immunorevealed. The amount of microsomal proteins (micrograms) applied was in order: rat liver 20, human liver 20, human granulocytes 20–40–80. On the left side, the size of molecular mass markers (in kilodaltons) is shown. A representative experiment of five is shown. (C) Microsomal vesicles from human granulocytes (0.3 mg protein/mL) were incubated in MOPS-KCl buffer (pH 7.2) at 37 °C. H6PDH activity was evaluated by measuring NADPH formation upon the addition of 1 mM $NADP^+$ and 10 μM glucose-6-phosphate (G6P) to microsomes. Microsomes have been subsequently permeabilized with Triton X-100 (T-X100, 1% final concentration) to allow the free access of the cofactor to the intraluminal enzyme. The product of lactonase activity, 6-phosphogluconic acid, was then measured enzymatically on the basis of NADPH formation by adding 6-phosphogluconate dehydrogenase (6PGDH). At the end of the incubation NADPH (2 μM) was added for calibration. A control experiment without G6P addition is also shown (bottom). A representative registration from six is shown.

the rate of apoptosis either in high (20 μM) or in physiological concentrations (data not shown).

4. Discussion

This paper demonstrates the presence of the complete G6PT–H6PDH–11βHSD1 system in the ER of human PMNs. Although G6PT protein and activity have been reported in our earlier papers [17,27], H6PDH protein and activity, as well as the occurrence of 11βHSD1 are shown for the first time in the ER compartment of PMNs.

The cooperation between H6PDH and 11βHSD1 based on their co-localization and their access to a common pyridine nucleotide pool in the ER lumen has been previously evidenced in rat liver and adipose tissue [3,4,28,29]. A major role of the G6PT–H6PDH–11βHSD1 triad is the prereceptorial activation of glucocorticoids, leading to metabolic effects in these organs and adipocyte differentiation in the latter. However, it seems that in other cell types the system may serve other purposes. The inhibition of G6PT in human and murine PMNs [17,30] and in gliomas [31–34] causes an increased apoptosis. The deletion of H6PDH induces skeletal myopathy with activated unfolded protein response in mice [35]. Therefore, the missing function of both G6PT and H6PDH impairs the viability of cells, possibly by altering the redox state of luminal pyridine nucleotides in the ER/SR.

Our present observations further strengthen this hypothesis. H6PDH activity was almost completely latent in PMN microsomes – similarly to liver and adipose microsomes – in accordance with its proposed topology, i.e. having the catalytic site in the lumen of the ER/microsomes. Moreover, the production of 6-phosphogluconate from G6P could be detected in PMN microsomes, in accordance with the reported dual activity of H6PDH possessing both G6P dehydrogenase and 6-phosphogluconolactonase domains. Similarly to H6PDH, 11βHSD1 activity was almost completely latent, which is again consistent with the proposed intraluminal topology of the enzyme. The latency of both activities is very likely due to the negligible permeability of the microsomal membrane to pyridine nucleotides [3,36]. As a consequence, the concerted action of the G6PT–H6PDH–11βHSD1 triad generates luminal NADPH and activates glucocorticoids in the granulocytes (Fig. 4A). Both the maintenance of the reduced state of NADPH and the prereceptorial glucocorticoid activation (see below) can be responsible for the antiapoptotic effects. In the absence of G6PT activity – due either to genetic or to experimental reasons – luminal NADPH production and the consequent cortisol formation should be impaired, which hampers the survival of the granulocytes (Fig. 4B). The observation that high, "metabolic" concentration of cortisol was needed in our experiments to overcome the proapoptotic effect of the G6PT inhibitor, while low, "pharmacological" concentrations were ineffective argues that a metabolic effect rather than a direct receptor-mediated

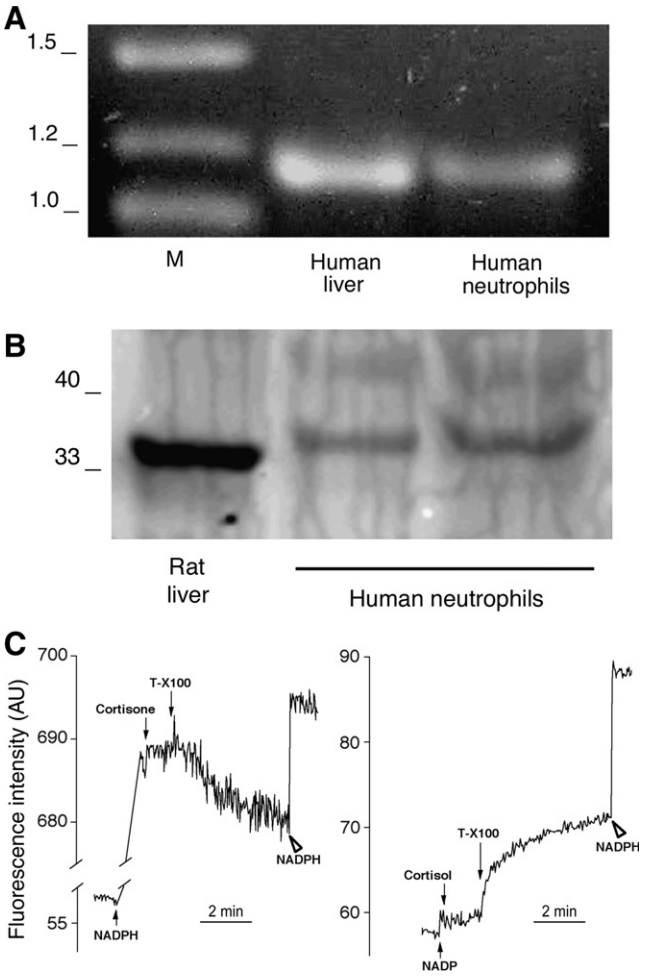

Fig. 2. Expression of 11βHSD1 at mRNA (A) and protein (B) level and 11βHSD1 reductase and dehydrogenase activities (C) in human PMNs. (A) Total RNA isolated from human neutrophils and human hepatic tissue (as a control) was reverse transcribed and subjected to PCR. A representative electrophoresis of the PCR products (revealed with ethidium bromide) of three is shown. The first lane (M) contains a mixture of DNA fragments of different size (in kilobase pairs). (B) Microsomes were prepared from human granulocytes and rat hepatic tissue (as a control), and microsomal proteins were separated by 12% SDS–PAGE. Gels were blotted on a nitrocellulose membrane and the 11βHSD1 protein was immunorevealed. The amount of microsomal proteins (micrograms) applied was: rat liver 100, human granulocytes 100 and 200. On the left side, the size of molecular mass markers (in kilodaltons) is shown. A representative experiment of five is shown. (C) Microsomal vesicles from human granulocytes (0.5 mg protein/mL) were incubated in MOPS-KCl buffer (pH 7.2) at 37 °C. The reductase (left panel) and dehydrogenase (right panel) activity of 11βHSD1 was evaluated by measuring NADPH consumption/formation upon the addition of 100 μM NADPH/$NADP^+$ and 20 μM cortisone/cortisol to microsomes. Microsomes have been subsequently permeabilized with Triton X-100 (T-X100, 1% final concentration) to allow the free access of the cofactor to the intraluminal enzyme. At the end of the incubation NADPH (5 or 3 μM, respectively) was added for calibration. Representative registrations from six experiments are shown.

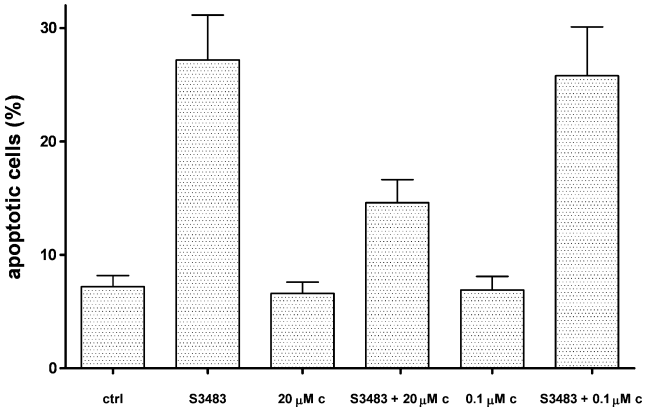

Fig. 3. Cortisol prevents the proapoptotic effect of the inhibition of microsomal G6P transport in human PMNs. PMNs (2×10^6) were incubated (at 37 °C in 5% CO_2/95% air for 60 min) in the complete RPMI 1640 culture medium. S3483 (200 μM) and cortisol (c; 20 or 0.1 μM) were added. Cells stained with annexin reagent (to reveal phosphatidylserine exposure on cell surface) and with propidium iodide were observed by a real-time confocal microscope. Apoptotic cells were identified on the basis of the phosphatidylserine exposure on the plasma membrane surface and on morphologic changes. A minimum of 200 cells was scored for each sample. Values are means ± S.E.M. of 3–5 independent experiments.

action is responsible for the prevention of the apoptosis. The assumption is further strengthened by the blockade of the anti-apoptotic effect of cortisol by the 11βHSD1 inhibitor carbenoxolone. On the other hand, cortisone – which can deplete luminal NADPH [3] – was not proapoptotic even at metabolic concentration. The possible explanation is that the relatively highly expressed H6PDH (Fig. 1) supported by a functioning G6PT can maintain the reduced state of luminal pyridine nucleotides by counterbalancing the activity of 11βHSD1 expressed at lower level (Fig. 2). The fact that cortisol added at high concentration can prevent the proapoptotic effect of the inhibition of G6PT demonstrates the functional cooperation of the G6PT–H6PDH–11βHSD1 system in the maintenance of the luminal NADPH pool in the ER of granulocytes (Fig. 4C). Interestingly, a similar model has been proposed in Leydig cells [37]. NADPH generated by the dehydrogenase activity of 11βHSD1 is utilized by 17βHSD3 involved in testosterone production. The cyclic reduction/oxidation of pyridine nucleotides in the ER lumen continues by coupling of 17βHSD3 and 11βHSD1 as long as their substrates are available.

Cortisol was used as an experimental tool to demonstrate the functioning of the G6PT–H6PDH–11βHSD1 system in the present work; its metabolic effect rather than hormonal actions were considered. However, cortisol produced by prereceptorial glucocorticoid activation may have a physiological role in the granulocytes. Locally activated glucocorticoids can exert their effects through transcriptional regulation of glucocorticoid-receptor target genes or by nongenomic glucocorticoid-receptor-dependent modulation of signal transduction pathways [38]. Glucocorticoids have been recently shown to prolong survival of human neutrophils, but the mechanisms and implications for leukocyte signaling and homeostasis are unclear [39–42]. The antiapoptotic effect of glucocorticoids at least in part can be exerted through the inhibition of Fas gene expression and downstream apoptosis signaling pathways [43]. Irrespectively of the mechanism, the decreased prereceptorial activation of glucocorticoids in the granulocytes of GSD 1b patients adversely affects the survival of these cells. Nevertheless, this effect has not been directly proven yet, and further work – eventually in G6PT [12] and H6PDH knockout mice [44] – is needed to clarify this point.

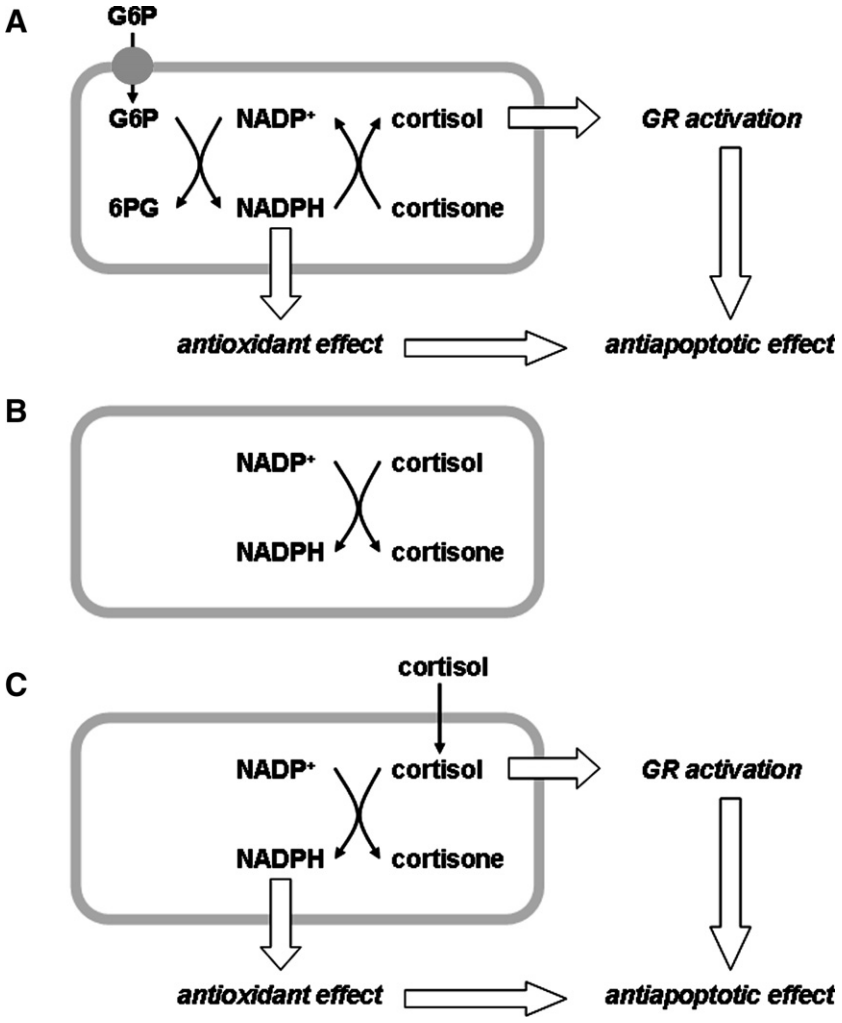

Fig. 4. Maintenance of luminal NADPH in the endoplasmic reticulum promotes the survival of human neutrophil granulocytes. (A) Under physiological conditions, intraluminal NADPH generation and glucocorticoid activation are ensured by the concerted action of the G6PT–H6PDH–11βHSD1 system. Both the maintenance of the reduced state of NADPH and the prereceptorial glucocorticoid activation lead to antiapoptotic effects. (B) Genetic deficiency or experimental inhibition of G6PT (or H6PDH) impairs $NADP^+$ reduction and cortisol production, abolishing the antiapoptotic machinery. (C) Cortisol addition through the action of 11βHSD1 restores the antiapoptotic mechanisms.

Taken together, our results demonstrate the existence of the G6PT–H6PDH–11βHSD1 axis in human PMNs at the first time. The concerted action of these proteins is responsible for the prereceptorial glucocorticoid activation, and also a major determinant of the redox state of the $NADP^+$/NADPH pool in the ER lumen. This system has been widely studied in the liver and in the adipose tissue, and its involvement in the pathogenesis of obesity, metabolic syndrome and insulin resistance has been proposed. Our results suggest that it also plays a role in the modulation of apoptosis and survival of PMNs.

Acknowledgements: We are grateful to Prof E. van Schaftingen and Dr. I. Gerin (ICP, Bruxelles, Belgium) for the H6PDH antibody. S3483 was kindly provided by Aventis Pharma Deutschland, Metabolic Diseases. This work was supported by grants of the Italian Ministry of University and Research (Grant No. RBAU014PJA), the Hungarian Scientific Research Fund (Grant Nos. F046740, T48939 and TS049851), the Hungarian Ministry of Health (ETT 182/2006) and by a Hungarian-Italian Intergovernmental S&T Cooperation Grant.

References

[1] van Schaftingen, E. and Gerin, I. (2002) The glucose-6-phosphatase system. Biochem. J. 362, 513–532.
[2] Nordlie, R.C., Foster, J.D. and Lange, A.J. (1999) Regulation of glucose production by the liver. Annu. Rev. Nutr. 19, 379–406.
[3] Piccirella, S., Czegle, I., Lizák, B., Margittai, E., Senesi, S., Papp, E., Csala, M., Fulceri, R., Csermely, P., Mandl, J., Benedetti, A. and Bánhegyi, G. (2006) Uncoupled redox systems in the lumen of the endoplasmic reticulum. Pyridine nucleotides stay reduced in an oxidative environment. J. Biol. Chem. 281, 4671–4677.
[4] Czegle, I., Piccirella, S., Senesi, S., Csala, M., Mandl, J., Bánhegyi, G., Fulceri, R. and Benedetti, A. (2005) Cooperativity between 11β-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase is based on a common pyridine nucleotide pool in the lumen of the endoplasmic reticulum. Mol. Cell. Endocrinol. 248, 24–25.
[5] Atanasov, A.G., Nashev, L.G., Schweizer, R.A., Frick, C. and Odermatt, A. (2004) Hexose-6-phosphate dehydrogenase determines the reaction direction of 11β-hydroxysteroid dehydrogenase type 1 as an oxidoreductase. FEBS Lett. 571, 129–133.

[6] Hewitt, K.N., Walker, E.A. and Stewart, P.M. (2004) Hexose-6-phosphate dehydrogenase and redox control of 11β-hydroxysteroid dehydrogenase type 1 activity. Endocrinology 146, 2539–2543.
[7] Bujalska, I.J., Draper, N., Michailidou, Z., Tomlinson, J.W., White, P.C., Chapman, K.E., Walker, E.A. and Stewart, P.M. (2005) Hexose-6-phosphate dehydrogenase confers oxo-reductase activity upon 11β-hydroxysteroid dehydrogenase type 1. J. Mol. Endocrinol. 34, 675–684.
[8] Seckl, J.R. and Walker, B.R. (2004) 11β-Hydroxysteroid dehydrogenase type 1 as a modulator of glucocorticoid action: from metabolism to memory. Trends Endocrinol. Metab. 15, 418–424.
[9] Draper, N. and Stewart, P.M. (2005) 11β-Hydroxysteroid dehydrogenase and the pre-receptor regulation of corticosteroid hormone action. J. Endocrinol. 186, 251–271.
[10] Tomlinson, J.W., Walker, E.A., Bujalska, I.J., Draper, N., Lavery, G.G., Cooper, M.S., Hewison, M. and Stewart, P.M. (2004) 11β-Hydroxysteroid dehydrogenase type 1: a tissue specific regulator of glucocorticoid response. Endocr. Rev. 25, 831–866.
[11] Kuijpers, T.W. (2002) Clinical symptoms and neutropenia: the balance of neutrophil development, functional activity, and cell death. Eur. J. Pediatr. 161 (Suppl. 1), S75–S82.
[12] Chen, L.Y., Shieh, J.J., Lin, B., Pan, C.J., Gao, J.L., Murphy, P.M., Roe, T.F., Moses, S., Ward, J.M., Lee, E.J., Westphal, H., Mansfield, B.C. and Chou, J.Y. (2003) Impaired glucose homeostasis, neutrophil trafficking and function in mice lacking the glucose-6-phosphate transporter. Hum. Mol. Genet. 12, 2547–2558.
[13] Kim, S.Y., Nguyen, A.D., Gao, J.L., Murphy, P.M., Mansfield, B.C. and Chou, J.Y. (2006) Bone marrow-derived cells require a functional glucose 6-phosphate transporter for normal myeloid functions. J. Biol. Chem. 281, 28794–28801.
[14] Yiu, W.H., Pan, C.J., Allamarvdasht, M., Kim, S.Y. and Chou, J.Y. (2007) Glucose-6-phosphate transporter gene therapy corrects metabolic and myeloid abnormalities in glycogen storage disease type Ib mice. Gene Ther. 14, 219–226.
[15] Herling, A.W., Burger, H.J., Schwab, D., Hemmerle, H., Below, P. and Schubert, G. (1998) Pharmacodynamic profile of a novel inhibitor of the hepatic glucose-6-phosphatase system. Am. J. Physiol. 274, G1087–G1093.
[16] Khan, A., Ling, Z.C., Pukk, K., Herling, A.W., Landau, B.R. and Efendic, S. (1998) Effects of 3-mercaptopicolinic acid and a derivative of chlorogenic acid (S-3483) on hepatic and islet glucose-6-phosphatase activity. Eur. J. Pharmacol. 349, 325–331.
[17] Leuzzi, R., Bánhegyi, G., Kardon, T., Marcolongo, P., Capecchi, P.L., Burger, H.J., Benedetti, A. and Fulceri, R. (2003) Inhibition of microsomal glucose-6-phosphate transport in human neutrophils results in apoptosis: a potential explanation for neutrophil dysfunction in glycogen storage disease 1b. Blood 101, 2381–2387.
[18] Kuijpers, T.W., Maianski, N.A., Tool, A.T., Smit, G.P., Rake, J.P., Roos, D. and Visser, G. (2003) Apoptotic neutrophils in the circulation of patients with glycogen storage disease type 1b (GSD1b). Blood 101, 5021–5024.
[19] Hjorth, R., Jonsson, A.K. and Vretblad, P. (1981) A rapid method for purification of human granulocytes using percoll: a comparison with dextran sedimentation. J. Immunol. Methods 43, 95–101.
[20] Csala, M., Braun, L., Mile, V., Kardon, T., Szarka, A., Kupcsulik, P., Mandl, J. and Bánhegyi, G. (1999) Ascorbate-mediated electron transfer in protein thiol oxidation in the endoplasmic reticulum. FEBS Lett. 460, 539–543.
[21] Margittai, É. and Bánhegyi, G. (2008) Isocitrate dehydrogenase: a NADPH-generating enzyme in the lumen of the endoplasmic reticulum. Arch. Biochem. Biophys. 471, 184–190.
[22] Ozols, J. (1993) Isolation and the complete amino acid sequence of lumenal endoplasmic reticulum glucose-6-phosphate dehydrogenase. Proc. Natl. Acad. Sci. USA 90, 5302–5306.
[23] Takahashi, T. and Hori, S.H. (1978) Intramembraneous localization of rat liver microsomal hexose-6-phosphate dehydrogenase and membrane permeability to its substrates. Biochim. Biophys. Acta 524, 262–276.
[24] Clarke, J.L. and Mason, P.J. (2003) Hexose-6-phosphate dehydrogenase: a bifunctional enzyme with broad substrate specificity and 6-phosphogluconolactonase activity. Arch. Biochem. Biophys. 415, 229–234.
[25] Ozols, J. (1995) Lumenal orientation and post-translational modifications of the liver microsomal 11β-hydroxysteroid dehydrogenase. J. Biol. Chem. 270, 2305–2312.
[26] Odermatt, A., Atanasov, A.G., Balazs, Z., Schweizer, R.A., Nashev, L.G., Schuster, D. and Langer, T. (2006) Why is 11β-hydroxysteroid dehydrogenase type 1 facing the endoplasmic reticulum lumen? Physiological relevance of the membrane topology of 11β-HSD1. Mol. Cell. Endocrinol. 248, 15–23.
[27] Senesi, S., Marcolongo, P., Kardon, T., Bucci, G., Sukhodub, A., Burchell, A., Benedetti, A. and Fulceri, R. (2005) Immunodetection of the expression of microsomal proteins encoded by the glucose-6-phosphate transporter gene. Biochem. J. 389, 57–62.
[28] Bánhegyi, G., Benedetti, A., Fulceri, R. and Senesi, S. (2004) Cooperativity between 11β-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase in the lumen of the endoplasmic reticulum. J. Biol. Chem. 279, 27017–27021.
[29] Marcolongo, P., Piccirella, S., Senesi, S., Wunderlich, L., Gerin, I., Mandl, J., Fulceri, R., Bánhegyi, G. and Benedetti, A. (2007) The glucose-6-phosphate transporter–hexose-6-phosphate dehydrogenase–11β-hydroxysteroid dehydrogenase type 1 system of the adipose tissue. Endocrinology 148, 2487–2495.
[30] Cheung, Y.Y., Kim, S.Y., Yiu, W.H., Pan, C.J., Jun, H.S., Ruef, R.A., Lee, E.J., Westphal, H., Mansfield, B.C. and Chou, J.Y. (2007) Impaired neutrophil activity and increased susceptibility to bacterial infection in mice lacking glucose-6-phosphatase-β. J. Clin. Invest. 117, 784–793.
[31] Belkaid, A., Copland, I.B., Massillon, D. and Annabi, B. (2006) Silencing of the human microsomal glucose-6-phosphate translocase induces glioma cell death: potential new anticancer target for curcumin. FEBS Lett. 580, 3746–3752.
[32] Belkaid, A., Currie, J.C., Desgagnes, J. and Annabi, B. (2006) The chemopreventive properties of chlorogenic acid reveal a potential new role for the microsomal glucose-6-phosphate translocase in brain tumor progression. Cancer Cell Int. 6, 7.
[33] Belkaid, A., Fortier, S., Cao, J. and Annabi, B. (2007) Necrosis induction in glioblastoma cells reveals a new "bioswitch function for the MT1-MMP/G6PT signaling axis in proMMP-2 activation versus cell death decision. Neoplasia 9, 332–340.
[34] Currie, J.C., Fortier, S., Sina, A., Galipeau, J., Cao, J. and Annabi, B. (2007) MT1-MMP down-regulates the glucose 6-phosphate transporter expression in marrow stromal cells: a molecular link between pro-MMP-2 activation, chemotaxis, and cell survival. J. Biol. Chem. 282, 8142–8149.
[35] Lavery, G.G., Walker, E.A., Turan, N., Rogoff, D., Ryder, J.W., Shelton, J.M., Richardson, J.A., Falciani, F., White, P.C., Stewart, P.M., Parker, K.L. and McMillan, D.R. (2008) Deletion of hexose-6-phosphate dehydrogenase activates the unfolded protein response pathway and induces skeletal myopathy. J. Biol. Chem. 283, 8453–8461.
[36] Bublitz, C. and Lawler, C.A. (1987) The levels of nicotinamide nucleotides in liver microsomes and their possible significance to function of hexose phosphate dehydrogenase. Biochem. J. 245, 263–277.
[37] Hu, G.X., Lian, Q.Q., Lin, H., Latif, S.A., Morris, D.J., Hardy, M.P. and Ge, R.S. (in press) Rapid mechanisms of glucocorticoid signaling in the Leydig cell. Steroids. doi:10.1016/j.steroids.2007.12.020.
[38] Löwenberg, M., Verhaar, A.P., van den Brink, G.R. and Hommes, D.W. (2007) Glucocorticoid signaling: a nongenomic mechanism for T-cell immunosuppression. Trends Mol. Med. 13, 158–163.
[39] Cox, G. (1995) Glucocorticoid treatment inhibits apoptosis in human neutrophils. Separation of survival and activation outcomes. J. Immunol. 154, 4719–4725.
[40] Meagher, L.C., Cousin, J.M., Seckl, J.R. and Haslett, C. (1996) Opposing effects of glucocorticoids on the rate of apoptosis in neutrophilic and eosinophilic granulocytes. J. Immunol. 156, 4422–4428.
[41] Heasman, S.J., Giles, K.M., Ward, C., Rossi, A.G., Haslett, C. and Dransfield, I. (2003) Glucocorticoid-mediated regulation of granulocyte apoptosis and macrophage phagocytosis of apoptotic cells: implications for the resolution of inflammation. J. Endocrinol. 178, 29–36.

[42] Nittoh, T., Fujimori, H., Kozumi, Y., Ishihara, K., Mue, S. and Ohuchi, K. (1998) Effects of glucocorticoids on apoptosis of infiltrated eosinophils and neutrophils in rats. Eur. J. Pharmacol. 354, 73–81.
[43] Chang, L.C., Madsen, S.A., Toelboell, T., Weber, P.S. and Burton, J.L. (2004) Effects of glucocorticoids on Fas gene expression in bovine blood neutrophils. J. Endocrinol. 183, 569–583.
[44] Lavery, G.G., Walker, E.A., Draper, N., Jeyasuria, P., Marcos, J., Shackleton, C.H., Parker, K.L., White, P.C. and Stewart, P.M. (2006) Hexose-6-phosphate dehydrogenase knock-out mice lack 11β-hydroxysteroid dehydrogenase type 1-mediated glucocorticoid generation. J. Biol. Chem. 281, 6546–6551.